National Institute of Dental Research

ANNUAL REPORT

Fiscal Year 1982

The document was prepared for administrative use at NIH. The comments and declarations of its contributors are their own and do not necessarily represent an official statement of the Institute.

National Institute of Dental Research
National Institutes of Health
Bethesda, Maryland 20205

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Part A

NATIONAL INSTITUTE OF DENTAL RESEARCH ANNUAL REPORT

Office of the Director

October 1, 1981 - September 30, 1982

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Dental Research Data Officer
National Institute of Dental Research
National Institutes of Health
Bethesda, Maryland

OFFICE OF THE DIRECTOR

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

October 1, 1981 - September 30, 1982

The National Institute of Dental Research is the primary sponsor of oral health research and related training in the United States. The Institute carries out its mission to improve the oral health of this nation by conducting and supporting a wide range of research programs in the areas of caries, periodontal disease, craniofacial anomalies, soft tissue stomatology and nutrition, restorative materials, and pain control and behavioral studies. Research is conducted through intramural laboratory and clinical studies, extramural grants and contracts, and the National Caries Program (NCP).

The Office of the Director determines the overall policy of the Institute and provides general management. All support services are incorporated under this Office, including planning and evaluation, scientific and health reports, financial management, personnel, equal employment opportunity, and research data and data processing activities.

Dr. David B. Scott, Director of the NIDR since 1976 and Assistant Surgeon General in the U.S. Public Health Service, retired December 31, ending a career that included 27 years of service in the NIDR. Dr. Scott was one of the original 13 staff members of the Institute, which was formally established by an Act of Congress in 1948. During his tenure as Director, he was responsible for overseeing 400 employees and a budget of $70 million, in addition to advising the Director of NIH and the Congress on progress in dental research.

Effective January 1, Dr. John F. Goggins, Deputy Director, NIDR, was appointed Acting Institute Director and continued providing leadership and coordination of NIDR policies and programs during the transition period of this fiscal year.

OFFICE OF THE SPECIAL ASSISTANT TO THE DIRECTOR

The Special Assistant to the Director was on reassignment this year to the Division of Health Policy Research and Education at Harvard University as Visiting Lecturer in Social Medicine and Health Policy, Harvard Medical School. The purpose was to identify dental research issues relevant for policy formulation and to address international aspects of biomedical and social/behavioral research, research manpower training, and the transfer of research results to health care systems. During that tenure, health science policy activities, both domestic and international, were initiated by this new Harvard-based unit, including studies of allocation of health research expenditures, credibility and fraud associated with health science research, academic-industrial relationships, and comparative analyses of allocation structures and processes for research and training in industrialized and developing countries. A cooperative venture between Fogarty International Center and the Health Science Working Group staff to analyze existing data obtained from OECD (Organization of Economic Cooperation and Development) countries appears to be at least one finite product emanating from this liaison relationship.

Substantial interview materials were gathered on the subject of international health policy issues relevant for Government and/or academic attention. These materials have been made available to both Harvard and the Fogarty International Center for program planning purposes.

Other activities of the Special Assistant during the year included:

Seminar presentations for the Harvard School of Public Health and School of Dental Medicine, University of Connecticut School of Dental Medicine, University of California-San Francisco Medical Center, Pan American Health Organization, Behavioral Scientists in Dentistry monthly seminar group.

Chair, International Relations Committee, International Association for Dental Research.

Consultant to Commission on Oral Research and Epidemiology, Scientific Program Committee, Federation Dentaire Internationale.

Consultant, World Health Organization Regional Office for Europe, Meeting on Planning Dental Services for Europe (Oslo, Norway).

Consultant, American Dental Association on Future of Dentistry, Committee on Dental Research, and Conference on Foods, Nutrition and Dental Health.

Program Consultation to School of Dentistry, University of Louisville, Sloan School of Management, Massachusetts Institute of Technology.

Conference Chair, Conference on Dental Research Needs and Opportunities in Africa (Lagos, Nigeria).

Planning Committee, New York State Bar Association International Conference on Population, Nutrition and Food Technology Transfer to Third World Countries.
Symposium organizer and chairman "Uses of Behavioral and Social Sciences in Dentistry," Federation Dentaire Internationale, (Rio de Janeiro, Brazil).
Session co-organizer and presenter, International Collaborative Research in Health, International Sociological Association (Mexico City, Mexico).
Presenter, Conference on Teaching of Behavioral Sciences in Schools of Dentistry, Case Western Reserve University (Cleveland, Ohio).
Presenter, Conference on Dental Hygiene Research (Winnipeg, Canada).
Reviewer, *Social Sciences and Medicines,* and *Journal of the American Dental Association.*

Publications appearing during FY 1982 included:

Ayer, Wm. and Cohen, Lois K., eds., "Some Social Aspects of Dentistry," Special Issue of Social Science and Medicine, Vol. 15A, No. 6, December 1981.
Cohen, Lois K. Book review of *Social Science Research and Decision-Making* by Carol H. Weiss and Michael J. Bucuvalas, N.Y.: Columbia Univ. Press, 1980, for *Health Services Research,* Fall 1982.
Bryant, P.S., Soble, R.K., and Cohen, L.K., eds., *NIDR Behavioral and Social Studies,* Bethesda, Maryland: USDHHS, NIH, NIDR, 1982.
Cohen, L.K. "Dentistry and the Behavioral/Social Sciences: An Historical Overview," Journal of Behavioral Medicine, Vol. 4, No. 3, pp 247-256, 1981.

PLANNING

Efforts continued in the coordination of the NIDR Long Range Research Plan FY 1983-87 and included: organizing and conducting a formal meeting reviewing the completed state-of-the-science papers in November with the Long Range Plan Coordinating Committee; finalizing the outline for the Plan in February; developing guidelines for and working with staff on summaries of the state-of-the-science papers (March-September), and assuring that all NIDR advisory bodies were kept informed of the Plan's progress. A science writer, Ms. Joan Wilentz, was recruited for three months to help with editing and preparation of the first draft. Ms. Karen Gross, our summer student employee from 1981, returned this past summer as a COSTEP and continued her literature review work on the epidemiology of selected oral diseases and conditions as well as initiating some review of relevant dental economic issues. Specifically, she prepared documentation for that part of the Plan that will address "the magnitude of the health problem."

This office again coordinated the development and preparation of the NIDR Research Plan, FY 1984-86, and delivered the document to the NIH Director in June 1982. A program review session was held in January 1982 with the Acting Director, NIH, to discuss the major advances and research plans of the Institute and its associated budgetary requirements.

Staff activities included providing the Budget Office with narrative documentation for various budget submissions, preparing briefing books for an orientation session with Congressman Waxman's staff in December as well as for the new NIH Director's orientation in April, and participating in the monthly Planning and Evaluation Officers' Committee meetings. As part of this latter activity, the Evaluation Officer was an active member of Subcommittees on Program Performance Summaries and a Basic/Applied/ Development Work Group.

EVALUATION

The Evaluation of the NIDR Craniofacial Anomalies Research Activities was completed and the report was distributed. The recommendations were reviewed and the following actions have been taken to date for implementation in FY 1984: emphasis will be placed on expanding molecular biology studies related to teratogenesis and wound healing; studies will be initiated on the regulation of the wound healing process, temporomandibular joint dysfunction in humans and animals, the major problems of individuals with acquired defects, growth modification techniques for the prevention of dento-facial deformities, and epidemiologic research on the need for and effectiveness of orthodontic treatment; and new training programs will be initiated in the interdisciplinary research of acquired craniofacial defects and molecular biology.

A task order contract with the Office of the Assistant Secretary for Planning and Evaluation was administered for a short descriptive study of the five NIDR Dental Research Institutes and Centers (DRIC). The purpose of this study was to collect available data from the files for a future evaluation of the DRICs. In addition to data needed to review the Program's goals and objectives, information was gathered on the budget and the distribution of DRIC projects relative to the six NIDR categorical program areas. Analysis of the data collected in this study should determine specific policy questions that will be the focus of a formal evaluation planned for FY 1983.

The 1983 NIDR Evaluation Plan was prepared and submitted for NIH and department review in May. NIH review sessions were held in July. The projects

approved for FY 1982 (the Evaluation of the NIDR Restorative Materials Research Activities and the Assessment of Selected Objectives Associated with Dental Research Institutes and Centers) were postponed to FY 1983 until the completion of the Long Range Research Plan. One additional project was submitted for review for FY 1983. This includes the Evaluation of Selected Oral Health Education and Promotion Activities of the National Caries Program.

Staff activities included informing the NIDR advisory bodies of evaluation activities, meeting with NIDR staff to prepare for future evaluation, monitoring existing evaluation projects, and serving on the NIH Evaluation Oversight Committee which meets monthly. The Evaluation Officer continued to be an active member of the NIH Centers Committee. This Committee reviewed existing NIH Centers as to their status, characteristics, evaluations, and is in the process of developing a report which will include considerations for future evaluation plans at both the BID and NIH levels.

LEGISLATION

This Office continues to provide liaison activities with the Division of Legislative Analysis, OD, NIH. This year the major activity included reacting to Congressman Waxman's bill, H.R. 6338, "a bill to amend the Public Health Service Act to revise and extend the authorities under that Act relating to national research institutes, assistance to medical libraries, and health promotion, and for other purposes." Also, other pieces of pertinent legislation were routed to Institute staff, and the NIDR Director was kept informed of the progress of health legislation affecting NIH.

OTHER ACTIVITIES

A variety of assignments were carried out for the Director, and included: preparing the Director's report to the National Dental Research Advisory Council and responding to requests for information on topics such as health survey research, clinical trials, long-term care research, and health risk assessment research. The Office has maintained the responsibilities for reporting prevention-related research, and, with the current thrust in this area, acts as NIDR Prevention Liaison in reporting to the NIH's Special Assistant to the Director for Research Related to Disease Prevention. In this latter activity, the Evaluation Officer serves on a Subcommittee on the Working Definition of Prevention which is developing a classification system for coding prevention-related projects. Consultation was provided during the year to researchers from the following institutions who requested advice: University of Illinois, University of California at Irvine, and University of Maryland. In addition, this office served as an assignment for an NIH Grants Associate.

Organizational and committee commitments honored during the year were:

Member, PHS Chief Dental Officer's Strategic Planning Committee.
Technical merit reviewer, proposals submitted to NHLBI, NIAID, NCI, and the VA (Geriatric Dentistry Residency Programs).
Coordinator, Behavioral Scientists in Dentistry monthly seminars.
Participant, NIH Grant Associate Seminar Series, September-June. Reviewer, *Journal of Dental Education.*
"Preventive Dentistry and Research at NIDR," lecture presented at Georgetown University School of Dentistry, April.
President, American Association of Women Dentists.
Prevention Committee, Dental Health Section, American Public Health Association.
Consultant to Membership Committee, American Association of Public Health Dentists.

OFFICE OF SCIENTIFIC AND HEALTH REPORTS

The Office of Scientific and Health Reports (OSHR), under the direction of Dr. Kenneth C. Lynn, Acting Information Officer, continues to serve the National Institute of Dental Research by implementing a versatile information program covering NIDR activities encompassing intramural and grant-supported research studies.

The OSHR bears primary responsibility for disseminating this information to audiences that include dental practitioners, the scientific community, members of Congress, educators, media representatives, and the general public. Through the development of a broad range of communications activities using NIDR and NIH information channels and direct media contact, the OSHR strives to increase awareness and understanding of the causes, prevention, and treatment of oral diseases and related disorders.

AUDIOVISUAL ACTIVITIES

The OSHR released a 30-second public service announcement for television this year entitled "Magical Munching," encouraging children to enjoy fresh fruits, nuts, and other non-decay promoting snacks as alternatives to sweet, sticky foods. This dental health message, distributed to 700 TV stations across the country, was telecast 2,300 times and viewed by over 133 million people.

OSHR staff also made arrangements to view five U.S. Department of Agriculture TV spot announcements on snacking that were part of a study to determine how

these commercials influence the eating patterns of young children.

During FY 1981, production of two films about the use of fluorides to prevent dental caries had been initiated by the OSHR in collaboration with the National Caries Program (NCP). Although it was anticipated that the films would be completed during FY 1982, further work on this project could not be continued because of the audiovisual and printing moratorium imposed by the Office of Management and Budget (OMB) in April, 1981. OSHR petitioned twice this year to exempt the films from the moratorium because of the urgent need to educate children and adults about the proven effectiveness of fluorides in preventing tooth decay, but both appeals were rejected.

An exhibit entitled "Prevention is the Key to a Lifetime of Dental Health," emphasizing the steps that can be taken to avoid tooth decay and gum disease, was developed by the Office and displayed at the health fair of the White House Conference on Aging in December 1981. During the Conference, convened to formulate a national policy on aging and to identify issues pertinent to the changing demographic trends of America's older population, OSHR staff monitored the exhibit and provided information about dental health to the Conference delegates. The NIDR exhibit is now permanently on display in the Visitor's Center of the Warren G. Magnuson Clinical Center.

The Office continued to coordinate the scheduling, storage, maintenance, repair, and procurement transactions for all NIDR exhibits. During FY 1982, these exhibits were shown at eleven meetings, including health educator, dental, and school board meetings. The OSHR also provided service necessary for the planning and completion of 12 new portable exhibits for the National Caries Program, and repaired and revised two other NCP exhibits.

Staff revised text for the NIH juke box on questions about periodontal disease, dental plaque, tooth decay prevention, fluoride, and malocclusion. The juke box is located in the Visitors Center of Building 31.

MEDIA

The OSHR continues to play a major role in serving as the contact point about NIDR activities and research advances for radio, television, and press representatives.

When the National Dental Caries Prevalence Survey was released early this year by the National Caries Program, the OSHR provided information about the nationwide decline of tooth decay in response to numerous media requests. This resulted in extensive press coverage, with articles about the survey appearing in the Washington Post, the Boston Globe, and the New York Times. Information about caries prevalence was also provided to syndicated columnist Sylvia Porter. In connection with the survey, Dr. James P. Carlos, associate director for the National Caries Program, was interviewed by KGNR of Sacramento, CA, and Janet A. Brunelle, chief of the NCP Biometry Section, taped an interview for the Voice of America. Two other radio stations - CILQ in Toronto and WASH in Washington, D.C. - also carried segments about the decline of dental caries.

The herpes simplex virus is another topic that generated wide media interest this past year. The OSHR furnished information and slides to Channel 9 for a "Morning Break" show that featured a discussion of this virus. Background material about herpes was also provided for inclusion in Dr. Art Ulene's medical segment of the NBC Nightly News and ABC's "20/20."

Other media coverage in which the OSHR was involved included publication of an article in the Wall Street Journal about the NIDR caries vaccine study and arranging for Dr. Carlos to be interviewed about sealants by radio station CHUN of Toronto.

In response to requests from free-lance writers and staff reporters of national magazines, the OSHR assisted in the preparation of articles about oral diseases and dental care by furnishing background information and photographs and reviewing copy. These articles appeared in publications such as *Better Homes and Gardens, McCalls, Vogue, Mademoiselle, Family Circle, Prevention, Changing Times, Spring, Children Today, Science Digest,* and *Science 82.*

The OSHR prepared 15 press summaries of papers delivered by NIDR scientists for use in the press room at the March meetings of the International and American Associations for Dental Research.

The Office maintains a close liaison with the American Dental Association by regularly submitting items of interest about NIDR intramural and grant-supported research for publication in the *Journal of the American Dental Association.* Another forum used to feature NIDR research of interest to physicians is the "From the NIH" column of the *Journal of the American Medical Association* (JAMA). This year, articles about NIDR work on aphthous stomatitis and the relation of taste sensitivity to age were published with the assistance of the OSHR. Information was also provided to JAMA for an upcoming article about orthodontics.

As a means of reaching science writers and media representatives about NIDR research advances,

programs, and activities, the OSHR also routinely contributes articles to NIH publications, including the *NIH Record, Search for Health* columns, and *News and Features.* This year NIH produced *Healthline,* a new research information periodical. The first edition was devoted to dental research, and included articles about canker sores, tooth decay, mouth cancer, and herpes basedon information provided by the OSHR. *Healthline* generated numerous requests from medical and dental professionals for additional information on the new toluidine blue rinse used in the detection of mouth cancer. This information was also provided to ABC TV nightly news.

PUBLICATIONS

The OSHR began extensive distribution of a new publication this year entitled "Snack Facts." This leaflet was one of six DHHS publications selected by the Office of the Assistant Secretary for Public Affairs for inclusion in the Department's health promotion initiative. "Snack Facts," along with other health campaign materials, will be sent to the ten PHS regional offices for their use in contacting media and community groups. Intended especially for children and their parents,the brightly-colored leaflet explains how sugary foods damage teeth and encourages children to enjoy snacks that will not promote tooth decay. It unfolds to a large poster that can be hung where children will be reminded of alternative between-meal snacks.

"Snack Facts" has been widely publicized through the TV spot announcement "Magical Munching," the Consumer Information Center in Pueblo, Colorado, and announcements appearing in professional and non-professional journals and magazines. Because of the overwhelming number of requests for this publication, OSHR received permission to reprint additional copies of the leaflet to meet the public's increasing demand for this dental health information.

Although the Office has submitted numerous documents requesting exemptions from the OMB printing moratorium in order to reprint several other Institute publications, permission has only been granted to reprint three - "Fluoride to Protect Your Children's Teeth," "Tooth Decay," and "Cleft Lip and Cleft Palate." Departmental approval was also given for two publications issued in cooperation with the National Caries Program to further their prevention efforts - "Fluoride Mouthrinsing in Schools...Protection for Children's Teeth," and "A Healthy Start...Fluoride Tablets for Children in Preschool Programs." Clearances for two new NCP fluoride posters - "Virtually Eliminate Dental Decay" and "Fluoride Isn'tJust for Kids" - are pending approval.

The OSHR assisted the National Caries Program and the Extramural Program with editing, printing, advertising, and distributing several issuances for their program activities. These include "Dental Caries Prevention in Public Health Programs," "Preventing Tooth Decay: A Guide for Implementing Self-Applied Fluorides in School Settings," "The Prevalenceof Dental Caries in United States Children," and "NIDR Behavioral and Social Studies." Staff also provided the NCP with assistance in publishing their program's history on the occasion of the NCP 10th anniversary.

In addition to providing information in leaflet form about oral diseases and prevention, this year the OSHR designed a one-page fact sheet as a new format for information materials. Two new fact sheets have been issued - "Fluoride to Protect Your Children's Teeth," and "Tooth Decay." Fact sheets on xerostomia, NIDR intramural research, and several others are also in preparation.

The Office revised a leaflet entitled "Sugar and Tooth Decay" for use by the NIH Nutrition Coordinating Committee. This publication is to be included in a series of NIH leaflets about health and nutrition that will be reproduced and distributed by large supermarket chains throughout the country.

In functioning as the clearance center for all manuscripts and abstracts written by Institute scientists and administrators, the OSHR processed 205 manuscripts and 153 abstracts. The OSHR also continued to obtain departmental clearance and provide editorial services as required for all other Institute publications.

The Institute publication, *NIDR Research News,* contained 25 science articles this year, plus additional items including notices about the availability of Institute publications, and appointments and awards of special note. This publication is sent to approximately 1500 dentists, members of dental societies, universities, members of the National Advisory Dental Research Council, the NIDR Programs Advisory Committee, the NIDR Board of Scientific Counselors, the NIDR Special Grants Review Committee, and science writers and editors of state and county dental journals who use these items in informing their readers about NIDR research and program activities.

NIDR Abstracts, which presents summaries of scientific papers published by NIDR investigators, contained 65 abstracts. This publication, designed to report research findings to the scientific community, is sent to libraries of dental schools and universities, members of the Institute's council and advisory committees, and U.S.

and foreign researchers. The dental section of the Pan American Health Organization also receives copies.

During FY 1982, announcements about Institute publications appeared in the Consumer Information Center directory, and other periodicals including the *Journal of Nutrition Education, Freebies For Kids, Free Stuff for Parents, Free and Inexpensive Learning Materials, National Enquirer, Help Yourself to Health* (a nationwide directory of health information and services by Art Ulene, M.D.), and newsletters of state dental societies. This publicity generated a large volume of requests for these publications from dental health professionals, members of Congress, State health departments, dental schools, nursing schools, hospitals, state, county, and community health agencies, coordinators of health fairs, and the general public.

The number of publications sent this past year in response to these requests totalled 617,903. Although most of these Institute publications were sent through a mailing service under contract with the OSHR, 50,000 copies of "Snack Facts" were distributed by the Consumer Information Center and 6,000 copies of "Radiation, Chemotherapy, and Dental Health" were distributed by the Government Printing Office.

EDITORIAL, PUBLIC INQUIRIES, AND OTHER ACTIVITIES

The OSHR was involved in the preparation and editing of various documents for use by members of Congress. Staff assisted in preparing the NIDR Director's Opening Statement for the FY 1982 Congressional Appropriations Hearings, editing testimony for both the House and Senate hearings, furnishing material on areas of promising research as requested by the NIDR Director for inclusion in the DHHS Secretary's report to Congress, and summarizing NIDR advances in diabetes, arthritis, cystic fibrosis, and digestive diseases for inclusion in the NIADDK's Special Reports to Congress about these diseases.

The OSHR also prepared a statement on dental caries prevention for the DHHS Secretary's use.

The Office continued the annual revision of portions of NIH publications describing NIDR programs. This resulted in the updating of appropriate sections of the *NIH Almanac, NIH Publications List, NIH Extramural Training, Scientific Directory and Annual Bibliography, Associate Training Programs in the Medical and Biological Sciences at the NIH,* and the Fogarty Center's annual report of *International Activities for FY 81.*

Staff prepared budget statements for the Office of Management and Budget, and, as requested, submitted plans reflecting a proposed five percent and ten percent reduction in printed and audiovisual materials. The Office also routinely provides audiovisual, budget, and administrative reports, and weekly reports of NIDR activities of interest to the DHHS Secretary.

This year, OSHR staff responded to 20,264 written inquiries (an increase of 34% over last year) and 1,137 phone calls from members of Congress, dentists and dental hygienists, Federal, State, and county health agencies, journals, professional organizations, and the general public. The majority of the written requests for information concerned such oral conditions and diseases as periodontal disease, canker sores and fever blisters, temporomandibular joint dysfunction, and xerostomia, plus additional requests for information about caries prevention.

The OSHR arranged for 143 American and foreign dental practitioners, dental students, and hygienists to tour the NIDR laboratories, and for NIDR staff to speak to various professional organizations.

OSHR reviewed chapters for a book entitled *The Over-the-Counter Drug Guide,* to be published this year by Harper & Row, and supplied photographs and text about NIDR programs for inclusion in *Opportunities in Dental Care,* a National Textbook Company publication. Staff also offered editorial assistance in preparing NIDR policy issuances for internal use, provided printing and editorial assistance in preparing the *NIDR Awards Book* and provided written copy for advertisements that appeared in professional journals inviting applicants to apply for the job as NIDR Director.

OSHR arranged for printing and photographic services for various Institute functions, and for the videotaping of television programs that highlighted NIDR research advances.

FLUORIDATION SPECIALIST

The Fluoridation Specialist, Mr. John Small, continued activities in support of public health officials in states and cities defending community water fluoridation in court cases initiated by persons or organizations opposed to fluoridation. This support has included developing communications among legal personnel involved in several cases, assisting in the preparation of affidavits and technical documentation, locating and briefing expert witnesses, transmitting current research findings and legal decisions to involved officials, performing liaison with other Federal agencies for information or expertise, and providing requested assistance to newsmen or journal writers publishing information on the proceedings and outcomes. During FY 1982, initial phases of cases in Illinois and Texas

were completed, and appeals are pending in cases in Pennsylvania, Ohio, and Illinois. Cases in Scotland and South Carolina are also continuing. All of these activities are carried out in close cooperation with the Centers for Disease Control's dental health staff and local health officials.

The Specialist served as a member of a five-person Ad Hoc Committee on Dental Fluorosis appointed by the Chief Dental Officer (CDO), USPHS, to review all available information on the prevalence of dental fluorosis attributed to the use of high-fluoride drinking water supplies. This review of past and current information was requested by the Administrator of the U.S. Environmental Protection Agency for that agency's use in reviewing the interim national drinking water regulations as they pertain to fluoride. The Committee completed its work and delivered a final report to the CDO in July 1982.

At the request of the Chief Dental Officer, whose office was initially established without any full-time staff, the Specialist performed several writing and documentation tasks for the CDO.

In January 1982, the Specialist noted, in regularly monitored information sources, indications of a continuing decrease in phosphate fertilizer sales and a growing unsold inventory of fertilizers. By April 1982, the possibility of significant reductions in the production of phosphate fertilizer and the fluoride compound byproducts used for fluoridation was apparent, and the Specialist undertook a telephone survey of producers, distributors, marketing reporters, and regulatory agencies to confirm this. The Specialist then alerted the Centers for Disease Control (CDC) about the probability of a fluoridation chemical shortage in future months, and assisted the CDC in establishing an alerting and reporting network of knowledgeable people so that the CDC dental health staff could monitor and influence the developing situation. Those arrangements remained in effect at the end of FY 1982.

The Specialist performed requested technical reviews of manuscripts for the U.S. Environmental Protection Agency, the American Dental Association, the National Academy of Sciences, the Centers for Disease Control, and the New York State Health Department.

The Specialist provided, in response to specific requests, sets of selected information on fluorides and health to the Canadian Dental Association, the Society for Epidemiologic Research, The Fluoridation Society (London), Time-Life books, several state and city health departments, and individual researchers. Mailings of current information were also made to about 100 foreign health officials and researchers on a special mailing key. Additional activities during the year included:

Presenting part of a continuing education course for health professionals given at Frederick Community College, Frederick, Maryland, in December 1981.

Attending and participating in an international symposium on fluorides and health at the Utah State University in May 1982.

Preparing a report on the status of community water fluoridation and water defluoridation for presentation at the FDI/WHO World Conference on Fluoridation in Vienna in October 1982.

Receiving computer training to develop a computer program aiding in the rapid retrieval of fluoride information materials used in responding to public inquiries.

FINANCIAL MANAGEMENT OFFICE

The Financial Management Office (FMO) continues to serve as the Institute's center for budgetary data and related activities and as the primary financial component of the Office of the Director. The FMO formulates budget estimates required to support operating and future programs, compiles budget justifications, and provides management controls over obligations and expenditures of funds. The FMO also advises the NIDR Director, Executive Officer, and program directors about the availability of obligation authority to carry out the Institute's initiatives.

During FY 1982, the FMO formulated zero-based budgets for prospective fiscal years and produced justification materials for the FY 1983 and 1984 budgets. Staff prepared budgetary estimates by mechanism and program areas reflecting changes from prior years for the Director's use in testifying at the Congressional hearings. The Office also maintained payroll records, generated monthly personnel status and program expenditure reports, tracked the funding of grants, requisitions, and purchase orders, and worked with Institute administrative staff to ensure that budgeted amounts were not exceeded, and that reprogramming actions were initiated in areas where additional funds were required.

The FMO continues to monitor the Institute's trans-NIH activities including research studies in the areas of diabetes, arthritis, nutrition, and disease prevention. The Office prepares special reports and grant forecasts and responds to requests for program and financial data from Congress, the Office of Management and Budget, and other Federal and non-Federal agencies.

In order to meet these increasing demands for the dissemination of financial data, FMO staff cooperated with the NIDR Word Processing Committee to develop new methods of using word processors to aid in data storage and the timely retrieval of information relating to the planning and execution of budget activities. In additon to updating current reporting methods, the FMO also formulated plans to electronically coordinate budget and program activities with other NIH components.

The FMO continued to contribute to the overall NIH resource pool of budget analysts and officers by actively participating in training programs for budget personnel. This past year, the FMO provided a Management Intern with a diversified, four-month training program.

PERSONNEL AND MANAGEMENT ANALYSIS SECTION

The Personnel and Management Analysis Section (PMAS) is the focal point for both the personnel and management analysis functions of the Institute. Personnel management activities encompass staffing and placement (including merit promotion), classification and pay management, employee relations, and employee development and training. Management analysis activities include providing staff advisory service and assistance on organizational and procedural problems, and serving as the central clearance and management point for Consultant Services, Conference Management, Contracting Out of Commercial/Industrial Type Product/Services, and Records Management.

During the past year, employee relations received the major emphasis within the personnel management operation because of changes made in the performance appraisal system for Merit Pay employees, the implementation of the Employee Performance Management System (EPMS), the establishment of new policies and procedures for cash awards, and the institution of significant alterations in the Federal Health Benefits Program.

In FY 1982, all Federal civil service employees came under one of three appraisal systems; the Senior Executive Service/Senior Scientific Service (SES/SSS); Merit Pay; or the Employee Performance Management System (EPMS). The SES/SSS system has been in effect for two years. The Merit Pay System was fully implemented and underwent several changes in policies and procedures, including the addition of the requirement that standards for three levels of performance be developed. The EPMS, the final link in the appraisal system legislated by the Civil Service Reform Act of 1978, was also activated. The EPMS applies to all Institute civil service employees who are not covered by the Merit Pay and SES/SSS systems. Throughout the year, group and individual meetings with managers and employees were held to delineate the three appraisal systems.

The Institute continued to have an active employee incentive awards program, incorporating several changes in Department guidelines and procedures. To help managers, the PMAS developed two issuances on incentive awards, one for quality increases and cash awards, and the other for cash awards for summer employees. Program chiefs, as well as Institute employees, were encouraged to submit names of nominees for the awards so that supervisors could continue to recognize the excellent quality of their staffs. For the second year, NIDR SES/SSS level staff was recognized for their contributions through bonuses at a percentage greater than the general Department approval level. These award activities culminated in another well-attended Annual Awards Ceremony.

Staffing activities received considerable attention again this year. The NIDR actively participated in an intensive program to help employees from the DHHS and Public Health Service adversely affected by budget and personnel ceiling cuts. In addition, the loss of several key NIDR officials, including the Director, Associate Director for Extramural Programs, and two branch chiefs, generated considerable staffing activity. All positions are at the Senior Executive level, and two of them - the Director and Associate Director positions - have involved nationwide searches. Such searches entail the establishment of positions and search plans (approved at the Department), search committees, and communication with over 150 professional societies and dental schools.

The Staff continues to collaborate with the NIDR EEO Officer and the NIDR EEO Advisory Committee on matters of joint concern. They actively participate in Advisory Committee meetings to keep the EEO community informed about Institute personnel policies, procedures, and activities. The staff also works closely with the NIDR EEO Officer and with managers in assuring the feasibility and legality of personnel activities related to affirmative action and EEO concerns. For a third year, minority and women summer hiring goals were exceeded through the cooperative efforts of managers, EEO, and the PMAS.

A number of new and revised NIDR Policy and Procedures were issued. New issuances included "Manuscript Clearance," "NIDR Incentive Awards - Special Achievement (Cash) Awards and Quality

Increases," "Cash Awards for Summer Employees," "Career Development Assignments for Normal Volunteer Patients," "Request and Approval for Acceptance of Payment of Travel Expenses in Cash or in Kind (HHS-348)," "NIDR Contract Policy," and "Essential Activities and Personnel." Revised issuances were "Clearance of Personnel for Separation or Transfer," "Acquisition of Consultant and Professional Services and Manuscript, Publication Costs and Reprints Without Covers," "Full Time Equivalent System," and "NIDR Training Policy."

Several reports which require Institute-wide response were coordinated by the Management Analyst. Both new and recurring requests were covered. These reports included the ADP Plan, NIH Organization and Functions Manual, Hardware Systems Narratives, Annual Survey of Records Holdings, Annual Report-Copying Equipment, Inventory of Word Processing Equipment, and Biennial Inventory of Controlled Substances.

Other areas of management analysis activity included completing the revised functional statements for the reorganization of certain intramural laboratories and with the abolishment of the Office of Collaborative Research, transferring its function and personnel to the Extramural Program. The use of NIH computer facilities was also increased to ease the workload in the PMAS, and was accomplished in part with the help of the Management Information Section in response to programming needs, and through the extended use of WYLBUR. The NIH 1167-1 (Authorization Notice) and NIH 1167-2 (Request for Delegation or Recision of Procurement Authority) were centralized in PMAS for securing approvals; and the PMAS also acted as coordinator in the installation and training needed to bring Delegated Procurement (DELPRO) on line.

During FY 1982, PMAS staff participated in several trans-NIH activities. These included membership and active involvement in the DPM Committee on the Continuing Education of Personnel Management Specialists, the DPM Professional Personnel Program Series, the NIH Committee to Develop Guidance on Implementation of the GS-560 Budget Officer Classification Series, the NIH Administrative Training Committee, and the NIH Office Technology Task Force.

DENTAL RESEARCH DATA OFFICER

The Dental Research Data Office (DRDO) serves the Institute as a specialized center for scientific and technical information related to current dental research. Through the collection, analysis, storage, and retrieval of subjective and statistical data, the DRDO is recognized as a unique source of information on dental research activities. This information is provided through regular publications as well as through individual subject matter reports.

Six printed reports are produced annually by this office: *National Institute of Dental Research Programs* (provides charts and tables which summarize and analyze the Institute's research grants, contracts, intramural research, training grants, and fellowship awards); *Dental Research in the United States and Other Countries* (a catalog of all reported ongoing dental research, classified by subject area); *Dental Research in the United States and Other Countries, Charts and Tables* (a supplement to the catalog); the *NIDR Annual Report* (an administrative requirement providing narrative material regarding Institute activities); *Selected List of Technical Reports* (a listing of dental-related technical reports that have been submitted to the National Technical Information Service (NTIS)); and *Dental Research Institutes and Centers* (includes research project summaries as well as charts and tables summarizing information on subprojects supported by each of the five multidisciplinary center (P50) grants).

As a result of a Departmentally imposed printing moratorium, fewer copies of *NIDR Programs* and *Dental Research* are available for distribution. These publications are now treated as inhouse documents, and the distribution policy has been revised to include only NIDR staff, dental libraries, regional libraries, and certain key persons within the dental research community. The practice of rewarding scientist registrants with free copies has been discontinued.

The abolishment of the Smithsonian Science Information Exchange (SSIE) during the last fiscal year has threatened the continuation of *Dental Research,* which has been produced in collaboration with SSIE since 1970. Research projects in progress reported to SSIE have been the primary source of information, and SSIE programming and cataloging procedures have provided tapes for conversion to camera-ready copy. The DRDO presently is consulting with the Management Information Section, NIDR, and the Research Documentation Section, DRG, to develop a new procedure for producing the catalog. Information on NIDR research will be provided from inhouse tapes, and representatives from the Army, Navy, and Veterans Administration are providing information on their dental-related projects. Reporting of other research, about four percent of the total, will depend on the initiative of individuals to send Notice of Research Project (NRP) forms to the DRDO.

The DRDO responds to dental research data requests from NIDR personnel and other users. During FY 1982, most of these requests (86%) have come from non-NIDR government sources. Requests have been primarily of a recurring nature and have dealt with such subjects as drug abuse, interferon, arthritis, nutrition, diabetes, drugs for rare diseases, Indian health, cancer, and aging. While the number of requests is somewhat less than it has been in the past, this is partially because more requests have been handled informally or through other channels.

Ties with the Office of Scientific and Health Reports (OSHR) are strengthened by the current organization. Since the Dental Research Data Officer is also Acting Chief of the OSHR, responsibilities can be divided according to the specialties of each office.

In addition to producing reports and publications, the DRDO is also involved in a variety of other information services, including conducting MEDLINE searches inhouse (aided by the installation of a CT-45 terminal in the OSHR), disseminating lists of NTIS Technical Reports to NIDR staff approximately bimonthly, and maintaining a library, including directories and indexes, that provides important references for staff.

An important function of the DRDO is the coding and indexing of research projects, to facilitate retrieval by subject. This process has been improved by the development of a data sheet and means of coding new grants. Important subjects can now be identified by the program chief at the time a grant is awarded, using the vocabulary of Medical Subject Headings (MeSH). Previously, MeSH terms have been used to code research contracts and intramural research projects only. This is an important supplement to the CRISP coding (Computer Retrieval of Information for Scientific Projects) already supplied by the Division of Research Grants.

Ongoing records of NIDR clinical trials are maintained and updated in the DRDO. Through the use of WYLBUR, a computerized record will make information on clinical trials more easily accessible.

A complete historical record of NIDR conferences and seminars was compiled in the DRDO. This record is stored on WYLBUR and will be updated to keep current information on Institute conference activities readily available.

Assistance has been provided to other offices in the preparation of NIDR publications. The Special Assistant for Research Manpower, Extramural Programs, NIDR, collaborated with the DRDO in compiling the booklet, *Graduate Training Supported by the NIDR*. Extensive use was made of WYLBUR Document Formatting capabilities, and storage on WYBLUR will facilitate updating of the booklet.

The Dental Research Data Officer serves as the Institute's Freedom of Information Act and Privacy Act Coordinator. By the end of the third quarter of fiscal year 1982, the same number of Freedom of Information Act (FOIA) requests had been received as the previous fiscal year. Requests most often are for applications, proposals, and progress reports for certain grants and contracts. Three comprehensive FOIA requests have been received, asking for information about several grants and contracts; one of these comprehensive requests was an updating of a previous request.

Privacy Act requests by the end of the third quarter of FY 1982 had increased by 25 percent compared to last year. All of the Privacy Act requests have been for grant summary sheets prior to Council meeting and for Clinical Center patient records. In the past year, one new Privacy Act System Notice has been developed (09-25-0152, Clinical Research) and other Notices have been revised for publication in the Federal Register.

Amendments to Department FOI regulations will affect changes in FOI procedures. There will be some decentralization of authority to the NIH level. Centralization within NIH has been proposed for record keeping and for handling certain types of requests. A new fee schedule will allow for greater recovery of costs incurred in responding to FOI requests.

The DRDO Technical Information Assistant, as a member of the NIDR Word Processing Committee, has helped to prepare the Questionnaire on Word Processing Activities and has assisted the Committee Chairperson in compiling special reports.

The Dental Research Data Officer, an active member of the Medical Library Association Dental Special Interest Group, presented a poster on "Dental Research in Progress - a Source" to the MLA meeting in Anaheim, California. The purpose of the presentation was to explain and highlight to librarians the multifaceted uses of *Dental Research in the United States and Other Countries.*

EEO PROGRAM

With the departure of the NIDR EEO Officer early in FY 1981 to serve as the NIH Federal Women's Program Manager, EEO functions were continued during FY 1982 under the direction of Garland N. Martin, Jr., Acting EEO Officer.

EEO Program activities encompassed support to minority schools, reports and analyses of the Institute's profile, the assignment of collateral EEO duties, and the recognition of EEO accomplishments. Other activities included a series of monthly educational seminars for employees, area meetings with employees, and EEO training for the EEO advisory Committee, staff, and Counselor.

DISCRIMINATION COMPLAINTS

The Institute had no informal or formal complaints of discrimination during FY 1982. The NIDR Counselor, at the direction of the NIH Division of Equal Opportunity, provided counseling in five cases for three other NIH Institutes. The EEO Officer, Equal Opportunity Assistant, and the Counselor provided assistance to employees whose concerns involved career counseling, job applications, leave, training, personal/family problems and supervisor/employee relations.

TRAINING

The NIDR continues its support of the NIH Minority Research and Training Programs through MBS and MARC and the NIH Extramural Grants Associate Program.

The NIDR EEO Advisory Committee received 2 ½ days of training about their role and responsibilities, presented by the Acting Associate Deputy Director for EEO, PHS. In conjunction with this training, the EEO Officer arranged for the publication of a reference/ course manual from material prepared by the PHS.

The EEO Officer, in cooperation with the EEO Advisory Committee, continued a series of monthly seminars on subjects of special interest to minorities and women. Included was a mini-series dedicated to the special health problems of women. The seminars will continue into FY 1983.

The Institute's Office Support Staff Training Activities Group (OSSTAG) developed a training session for NIH secretaries as part of the Secretaries Week Program of the NIH. The session, entitled "Success for Today and Tomorrow," was presented by Current Office Concepts, an outside firm.

The Institute provided training to the EEO Officer, EO Assistant, Counselor, Delegate and Alternate to the NIH Woman's Advisory Committee, and Representative and Alternate to the NIH Handicapped Employees' Committee in their areas of responsibility.

The NIDR EEO Officer analyzed and is continuing to monitor the NIDR Training Plan, which went into effect in FY 1981. Training plans were prepared in FY 1982 for all NIDR employees. This program is a major step in the upward mobility and full utilization of NIDR employees.

NIDR employees continued to enroll in the NIH Career Education Center. The Institute also actively participates in the NIH Management Intern and Stride programs, as well as other upward mobility programs of the DHHS and the NIH.

RECRUITMENT AND SELECTION

The EEO Office extended its list of contacts at minority and women's schools to over 300 and added a large number of minority affairs officers at non-minority schools to the list. In FY 1982, the EEO Office, in cooperation with the EEO Advisory Committee, sent over 225 packets of information to these contacts concerning the NIDR mission and the summer program of the NIDR. This network of contacts has been computerized to allow easy retrieval, changes, and additions.

The NIDR EEO Officer and the Director of Intramural Research participated in an Affirmative Action Recruitment Conference held at the NIH. The Conference established communication with minority student affairs coordinators at non-minority schools across the country.

The NIDR, as part of its Civil Rights program, sent both the Institute's EEO Officer and a scientific investigator to the annual MBRS Symposium in Albuquerque, New Mexico. The two representatives were sent on behalf of the Institute's Scientific Director to strengthen ties with the MBR schools, students, and faculties, and to recruit for the Institute.

The EEO Assistant distributed recruitment information at the Federally Employed Women's Conference in San Antonio, Texas.

NIH VISITING PROFESSOR PROGRAM

The NIH Visiting Professor Program was set up to stimulate minorities to choose a career in biomedical research, attract some minorities to NIDR intramural programs, and make minority schools more aware of opportunities in biomedical research at the NIH. The program will enable the schools' faculty and students to learn from members of the NIH/NIDR intramural staff. These staff members will spend from one week to one month at the schools, lecturing or teaching in their area of expertise.

Although the NIDR is one of the smallest Institutes o the NIH, I3 scientists have agreed to participate in the program. This is proportionally a greater number than

most of the other NIH BIDs. The scientists' specialties include areas such as connective tissue, chemotaxes, immunology, complex carbohydrates, nuclear magnetic resonance, enzymology, peptide chemistry, autoimmune disease, and herpes simplex virus.

EMPLOYEE MEETINGS

A series of area meetings started in FY 1981 to communicate information about the EEO Program and to determine employee concerns is scheduled to be completed in September 1982. These meetings are held by the EEO Advisory Committee, in cooperation with the Acting EEO Officer and NIDR management. A summary encompassing all the individual area reports will be prepared for the Director, NIDR.

MULTI-YEAR AFFIRMATIVE ACTION/FEORP PLAN

The EEO Officer, in cooperation with NIDR management officals, prepared the 1982-1986 Multi-Year Affirmative Action and Federal Equal Opportunity Recruitment Plan for the Institute. The plan included an underrepresentation analysis, work force profile, listing of priority recruitment targets (and criteria), plans for the prevention of sexual harassment, and an outline for the implementation of FEORP.

NATIONAL CONFERENCES AND MEETINGS

The Institute's EEO Officer and a scientist from the Intramural Program attended the MBRS Symposium in Albuquerque, New Mexico; the EO Assistant and the Delegate to the NIH Women's Advisory Committee attended the Federally Employed Women's Conference in San Antonio, Texas; the EEO Officer and the NIDR EEO Counselor attended the Blacks In Government Conference in Washington, D.C.; and the EEO Officer attended the Bureau of National Affairs Conference on "EEO in the Federal Sector" and The 39th Joint Annual Meeting of Beta Kappa Chi/National Institute of Science.

The NIDR EEO Officer, Executive Officer, Personnel Officer, and Budget Officer attended the National Public Health Service Equal Employment Opportunity Conference held in Reston, Virginia. The EEO Officer was an invited speaker for one session which was attended by all participants - "Managing Your Diverse Workforce: Understanding Differences".

OTHER PROGRAM ACTIVITIES

The assignment of collateral duties in EEO to the NIDR staff includes the appointment of the EEO Counselor, the Delegate and Alternate to the NIH Women's Advisory Committee (WAC), the Delegate and Alternate to the Handicapped Employee Committee, and the Representatives to the EEO Advisory Committee. The Delegate and Alternate to the Women's Advisory Committee and half of the members of the EEO Advisory Committee were appointed in FY 1982. The Delegate to the NIH Handicapped Committee was reappointed.

Two NIDR employees received the Institute's EEO Achievement Award for their outstanding contributions to the NIDR EEO Program. Two other employees received the EEO Advisory Committee's Certificate of Appreciation for their efforts in EEO. The EEO Officer and the EO Assistant received certificates from the NIH for their work with the NIH Hispanic Program. The EO Assistant received the BID EEO Special Achievement Award for her work with NIH/NIDR handicapped employees.

Publication of the new NIDR EEO Report began in FY 1982. The report will continue to be published quarterly. In addition to containing information about the NIDR EEO Program, the EEO Report includes articles from the Personnel Officer and the NIDR Safety Committee, as well as national EEO news of interest.

MANAGEMENT INFORMATION SECTION

The Management Information Section (MIS) is involved in the application of computer technology in the performance of NIDR research and administrative activities. MIS staff has continued the development of the Research Project Management System (RPMS), a collection of separate files dealing with grants, contracts, dental research subprojects and intramural projects. The system allows for the retrieval of information on these subjects that is needed for programmatic, management, and public use. Files can be accessed both separately or jointly via terminals linked in the batch mode to DCRT.

Information concerning program management and direct operations is provided by using files generated by the Division of Financial Management in the form of monthly magnetic tapes. Report production stems from both the Allotment Ledger Master File and the Open Document File. NIDR management and budget officials use these reports for budget tracking and reconciliation purposes.

In addition to the existing files in the Research Project Management System, a number of additions and enhancements have been made during FY 1982.

The MIS developed a computer-based Full Time Equivalency Tracking System that provides timely personnel ceiling balance information and fiscal year projections on a recurring basis. Data is collected from timekeeper forms and input using a Command

Procedure program developed by the MIS staff. Programs are then submitted by another Command Procedure which automatically produces the required number of copies for the various recipients. The entire procedure can be completed in a few hours, providing management with a near "real-time" FTE information capability.

The NIDR Travel File also was enhanced; final report production and distribution are handled by another Command Procedure. The travel file has proven to be extremely useful to NIDR Extramural Program staff as an effective means of accounting for travel commitments and obligations.

During the last fiscal year, the NIDR has served as one of the test institutes using the DRG-developed system for the in-house generation of grant award statements. MIS staff, working in conjunction with DRG and the NIDR Grants Management Office, successfully implemented this series of programs which has proven to be an extremely efficient and cost-effective manner of producing award statements. The time savings realized represent a reduction from approximately three days to fifteen minutes. The cost to produce an award, once estimated at three hundred dollars, is now three dollars for computer time.

Future plans call for the in-house production of all award statements, the obligation of funds locally, and inclusion of the indirect cost on the award statement. The data that are input on the award statement provide an update transaction file for use by DRG in their daily update to the DRG Open/Pending Files, thereby reducing the "time-lag" problem.

The NIDR Word Processing Committee, formed last year, has gone forward with a recommendation to purchase a NBI word processor for the NIDR Extramural Programs. Future plans include networking the two existing NBI's (Buildings 30 and 31) with the one in the Westwood Building, either directly or through WYLBUR. This will reduce time spent awaiting receipt of documents and will allow for use of the WYLBUR electronic mail facility.

Another file created by MIS staff is the Telephone Directory File, which not only allows for customized telephone directories by building, name, room, etc., but also aids in the production of mailing labels for various distribution lists. In addition, this file can be linked with the NIDR TAPS Personnel File and the NIDR FTE Tracking System for shared information report production.

Part B

NATIONAL INSTITUTE OF DENTAL RESEARCH ANNUAL REPORT

National Caries Program

October 1, 1981 - September 30, 1982

NATIONAL CARIES PROGRAM

NATIONAL INSTITUTE OF DENTAL RESEARCH

OCTOBER 1, 1981 - SEPTEMBER 30, 1982

REPORT OF THE ASSOCIATE DIRECTOR

Following the demonstration in FY 1981 that widespread use of fluorides has caused a measurable reduction in caries prevalence among children, efforts have increased to find additional ways to deal with the high levels of disease still occuring at these ages and among the older segments of the population.

During the past year, major advances were made toward understanding the complex ecology of dental plaque and its role in the initiation of caries. NCP grantees reported that plaques of caries-resistant individuals have lower proportions of *Streptococcus mutans* and lactobacilli, and higher levels of *S. sanguis* and *Veillonella*. In addition, oral microflora in these individuals appear less able to metabolize sucrose to acids with low disassociation constants. Saliva from caries resistant subjects also contains protein fractions which do not favor growth of *S. mutans* and which adsorb to enamel and may interfere with bacterial colonization. Other grantees demonstrated that adherence of bacteria to enamel is a process that is biochemically distinct from that of bacterial aggregation, a presumably desirable phenomenon if it can be induced before adhesion occurs. It is now believed that salivary glycoproteins play a key role in these processes.

The possibility that a non-cariogenic, "safe" oral flora might be established and maintained, continued to receive attention. Several investigators began experiments with recombinant DNA in an attempt to identify specific bacterial genes which mediate cariogenicity. Other NCP-supported grantees produced mutant forms of *S. mutans* which are deficient in lactate dehydrogenase, and have decreased cariogenic potential in laboratory animals. Efforts also continued to isolate and purify the antigenic components of cell walls of oral streptococci. Several such antigens were successfully used, with and without adjuvants, to induce a secretory IgA response in rats, and to partially protect the animals against caries.

Staff scientists of the NCP reported the results of an epidemiologic survey of dental fluorosis which suggested that, despite the ubiquity of fluoride in the modern environment, the prevalence of fluorosis has not increased. As dental fluorosis is the most sensitive available indicator of excessive fluoride intake, such studies provide a means for periodic monitoring of fluoride ingestion.

The decrease in dental caries among children is an encouraging observation, which attests to the success of recent research on caries prevention. To increase this trend it is critical to make fluorides, in a variety of delivery systems, available to more children, as well as to sustain those preventive programs already in existence. To this end, the NCP continued to expand efforts to promote these programs by a variety of methods, including films, posters, brochures and numerous lectures and seminars by staff members. With very limited resources, we are attempting to encourage self-applied, school-based fluoride programs for the approximately 35 million children not yet involved.

A continued decrease in loss of teeth among children does, however, raise several additional problems. Inevitably it will result in more teeth at risk to caries among adults and the aged. In this regard, caries of the root surfaces of teeth appears to be a problem of particular concern. Accordingly, the Program has developed plans for a comprehensive effort to investigate the prevalence, etiology and prevention of dental caries among older segments of the population. Thus, new target groups will require attention, if caries is to be eventually brought under control.

In July, the NCP hosted the annual Congress of the European Organization for Caries Research in Annapolis, Maryland, as that organization met in the U.S. for the first time to acknowledge ten years of close scientific collaboration with us.

Several staff changes were made during the year. Dr. Ralph A. Frew was appointed Acting Chief, Caries Prevention and Research Branch, replacing Dr. William Bowen, who left the Program. Dr. Michael F. Cole was appointed Acting Chief, Etiology Section and Dr. H. M. Stiles became Chief of the Preventive Methods Development Section.

The following narrative sections summarize the activities of the National Caries Program during FY 1982, in each of our four R&D Strategy Areas.

STRATEGY AREA I. Combatting the Microbial Agent

MICROBIAL AND BIOCHEMICAL STUDIES OF PLAQUE AND SALIVA

Numerous suggestions have been made to explain the apparent resistance of some individuals to dental caries. One suggestion is that the oral microflora of caries-resistant individuals differs from that of caries-susceptible individuals. Evidence supporting this suggestion appears in studies showing that caries-associated dental plaque harbors higher concentrations of *S. mutans* and *lactobacilli* and lower concentrations of *S. sanguis* than plaque on nondiseased dental surfaces.

A few of these studies, such as one conducted last year at the University of Maryland, have attempted to compensate for different dietary patterns of the subjects. In the Maryland study each subject was fitted with an intraoral prosthesis supporting a small slab of bovine enamel exposed to saliva. The prosthetic devices were immersed several times daily for 14 days in either a test substrate containing sucrose or a control substrate of saliva only. Periodically, segments of the appliances were removed for quantitation of plaque bacteria. The quantitation of the responses of several types of oral bacteria to longitudinal dietary substrate challenges revealed marked differences in the caries-resistant and susceptible individuals. In the resistant individuals: 1) the proportion of *S. mutans* to total streptococci was lower; 2) the proportion of *V. alcalescens* to total anaerobes was higher; 3) there was a consistent and substantially larger ratio of *S. mutans* to *V. alcalescens* when measured over a period of seven days; and 4) plaques harbored higher levels of *Veillonella* species, gram negative anaerobic rods, *S. sanguis*, and *Neisseria* species, and lower levels of lactobacillus species than plaques from caries susceptible mouths. Enamel slabs carried by resistant subjects also showed decreased decalcification and the plaque on the slabs formed less lactic acid when incubated with sucrose than did comparable plaques from caries susceptible individuals.

Salivary *S. mutans* levels were found to reflect the differences in plaque *S. mutans* levels. In the susceptible group, the mean *S. mutans* count was 2.1 x 10^5 per ml, representing 0.42% of the total streptococci in the saliva. In the resistant group, the mean count was 4.3 x 10^3 per ml, representing only 0.03% of the total streptococci.

Another grantee has explored plaque acidogenesis in the caries-resistant and susceptible populations. The investigator found that the plaque pH minimum after sucrose challenge was significantly lower in the susceptible than in the resistant group (6.1 ± .3 vs. 7.3 ± 0.8 respectively) though there was no difference in the resting plaque pH of the two groups. The investigator reports that the relatively modest formation of acid following a sucrose rinse in resistant subjects, appears to be due to a decreased ability of their plaque to generate low pK acids such as lactic and formic acid. He also notes that when salivary access is reduced, the pH minima of resistant plaques becomes similar to that of susceptible plaques, suggesting that saliva exerts a strong influence on plaque pH.

Observations on the importance of low pK acids are consistent with the information that *S. mutans* is a good producer of these acids, and that there are higher proportions of *S. mutans* in susceptible than resistant plaques and are consistent with recent findings by a grantee that lactic acid dehydrogenase deficient mutants of *S. mutans* have low cariogenicity. The inability to form lactate in these mutants leads to an accumulation of pyruvate which probably is rapidly converted to acetic and other weak acids.

To explore possible salivary effects on the microbial composition of plaque, the grantees examined parotid and submaxillary saliva samples from resistant and susceptible subjects. They found that saliva from resistant subjects contains protein fractions, which support less growth of *S. mutans* and *S. sanguis* than similar factors from susceptible subjects. Previous studies also have shown that several of the proteins in the fractions bind calcium and adsorb to hydroxyapatite (HA) and hence may affect colonization of bacteria to oral surfaces.

Together, these experiments reveal consistent differences in plaques obtained from the two populations, with plaques from caries resistant subjects exhibiting less cariogenic potential than those from caries susceptible subjects. The underlying mechanism responsible for the difference is presently not clear.

ENVIRONMENTAL CONDITIONS AFFECTING BACTERIAL GROWTH

Several grantees are studying the composition and structure of the surfaces of the oral streptococci to obtain information on the interactions of these microorganisms with tooth surfaces, other bacteria, oral mucosal membranes and with molecules such as salivary glycoproteins. By means of continuous culture techniques, the scientists found that conditions under which oral bacteria are grown have a profound effect on some, but not all components of the surface and on metabolic activities, including the production of acid and other excreted products. Thus, though cells grown at different generation times and at a variety of pH

values showed little variation in the polysaccharide-peptidoglycan complex, wall polymers were greatly affected as were the relative amounts of extracellular products such as lipoteichoic acid (LTA), capsular polysaccharides, and extracellular proteins.

The continuous culture technique was also used to study the effect of nutrients on lactic acid formation by *S. mutans*. The grantees have established that the intracellular level of fructose I, 6-diphosphate (FDP) is an important regulator of acid production in *S. mutans*, as it is in many other anaerobes. In *S. mutans*, glucose metabolism occurs primarily by glycolysis. During this process, the concentration of intracellular FDP that is formed is dependent upon the availability of glucose. Under glucose-excess condition, the concentration of intracellular (FDP) is sufficiently high to activate lactate dehydrogenase (LDH), at or near full capacity, and therefore rapidly produce high concentrations of lactic acid. Under glucose-limited conditions, the concentration of FDP is low and corresponding levels of lactic acid are produced.

Several studies were done to elucidate the effects of sucrose-containing diets on the oral microflora. Results show a significant influence by dietary sucrose on the concentration of certain species of bacteria in some or all intraoral sites. Furthermore, sucrose metabolism is higher in salivas of caries susceptible children (4-8 years of age) than in salivas of caries resistant children of the same age.

MECHANISMS OF ADHERENCE AND AGGREGATION

Saliva contains a number of high molecular weight glycoproteins which agglutinate certain oral streptococci. Evidence suggests that these "agglutinins" have two seemingly conflicting roles: first, they form a pellicle on teeth that promotes bacterial attachment and second, they aggregate salivary bacteria and promote their clearance from the oral cavity. Both activities are being studied at the present time.

Scientists supported by the Program have established that agglutination of *S. sanguis*, but not *S. mutans*, requires the presence of sialic acid in the salivary glycoprotein. In contrast, adsorption of bacteria to pellicle does not require sialic acid in the pellicle glycoprotein. Thus, salivary factors which promote aggregation appear to be different from those which promote adherence. These factors may play a role in caries resistance. In studies of this phenomenon, scientists discovered greater saliva-mediated adhesion activity and lower aggregating activity among caries susceptible individuals. Other investigators have found that adherence of *S. mutans* to pellicle is inversely related to salivary glycoprotein concentration, suggesting that glycoproteins specifically adsorbed to enamel act as receptors for *S. mutans*, whereas excess proteins or glycoproteins in solution specifically interact with the bacterial ligand to inhibit adherence.

The role of sucrose-derived water soluble and insoluble glucans in the attachment of *S. mutans* to smooth surfaces is becoming clearer through recent experiments using lectins (plant glycoproteins with strong agglutinating properties) as specific reagents. The results showed that lectins, which interact with glucans, do not block initial attachment of *S. mutans* to hydroxyapatite (HA) whereas a lectin, which interacts strictly with cell surface proteins, does block initial attachment. The latter lectin did not remove *S. mutans* cells already attached to the surface, and did not markedly inhibit sucrose-mediated adherence of *S. mutans* to a layer of *S. mutans* cells already bound to the surface. The primary attachment reaction is now thought to be sucrose-independent and involve proteins of the cell surface, whereas colonization of *S. mutans* involves cell-to-cell interactions mediated by sucrose-derived glucans.

The distinction of aggregation and adherence is clearly seen in certain mutants of *S. mutans* that are defective in the ability to form glucans. When the mutants were cultured in a sucrose-containing medium, there was an increase, relative to the wild type, in the synthesis of water-soluble, extracellular glucans, and a decrease in the water-insoluble, cell associated glucans. This was associated with decreased adherence of the mutants, relative to that of the wild type, to smooth surfaces. However, the ability of the sucrose-grown cells to agglutinate was not significantly different.

Scientists supported by the Program are attempting to determine the nature of the bacterial protein and the host salivary receptors involved in adherence. Recently they have isolated a protein component from the surface of *S. sanguis* that competitively blocks adherence of the microorganism to saliva-coated HA. The protein appears to bind to the salivary pellicle at the sites to which *S. sanguis* would bind. Blocking occurs at concentrations of the protein which do not grossly affect the growth of *S. sanguis* and *S. mutans* in culture.

Scientists also are studying the interactions of different bacteria. These interspecies reactions must be quite specific because a small number of associations between particular species, such as that between *S. sanguis* and *Actinomyces viscosus*, are fairly common in plaque. In research on this phenomenon, scientists have established that: I) coaggregation requires the interaction between a protein/glycoprotein (i.e. lectin) on *A. viscosus* TI4V with a carbohydrate on S. sanguis

34; 2) the interaction is specifically inhibited by lactose, certain other β-galactosides, and various anionic compounds (e.g. sodium dodecyl sulfate), which all react at or near the same site on the TI4V lectin; and 3) a crude, water-soluble carbohydrate preparation has been obtained from *S. sanguis* 34, which inhibits the coaggregation between *S. sanguis* 34 and *A. viscosus* TI4V more effectively than does lactose.

GENETICS OF ORAL BACTERIA

Several scientists supported by NCP are developing systems to identify and manipulate the genes in the oral streptococci. Such recombinant DNA systems can be used to improve our understanding of how these streptococci cause oral diseases, and can be used to decrease the cariogenic potential of the oral microflora, for instance, by maintaining immunity against virulent strains of microorganism, or by leading to bacterial antigens for use in an anticaries vaccine.

In brief, the recombinant DNA technique involves removing identifiable sequences of genes from specific donor bacteria, adding them to receptor bacteria, and observing the resultant changes in the bacterial characteristics. The procedure involves cutting chromosomes at specific places, isolating the dissected gene sequences *in vitro*, attaching these gene sequences to carriers, and inserting the carriers into new cells. All of these techniques have been developed in the last few years and are now used extensively in microbial research. The carriers that are most frequently used are the short lengths of DNA, called plasmids, often found in closed loops, that are not part of the regular chromosomes of many bacteria. Initial research efforts by NCP grantees centered on determining if *S. mutans* and other oral streptococci contained these extra-chromosomal elements. In general, no plasmids of clinical importance were found in any oral streptococci. However, it was found that plasmids, which confer resistance to the commonly used antibiotic, erythromycin, may occur naturally in the oral streptococci. Furthermore it was found possible to introduce purified plasmids into *S. sanguis* (strain challis).

Scientists plan to use recombinant DNA techniques to establish the relative importance of specific genes in cariogenicity. It may thus be possible to genetically construct strains of *S. mutans* which are noncariogenic, but are still strongly able to colonize the oral cavity, and in so doing, prevent infection or even supplant a cariogenic microflora. The use of recombinant DNA systems may also provide a means for obtaining large amounts of candidate antigens (e.g. GTF) for use in an anticaries vaccine.

STUDIES WITH BACTERIAL MUTANTS

In the last year scientists, through studies with *S. mutans*, have added new information on the genetic basis for caries virulence of this microorganism. Several investigators have isolated and characterized mutants that have reduced potential to initiate caries. Nevertheless, the mutants produce acid, adhere to glass, and colonize normally in the oral cavities of rats. Other mutants have decreased cariogenic potential, due apparently to defects in other traits, such as lactate dehydrogenase (LDH), aggregation, plaque formation, and adherence. In several experiments, it was shown that mutants could complement each other's defects so that mixed infections were more virulent than infections by only one mutant.

Some of these mutants have been used successfully to replace wild type *S. mutans* in rat and monkey models and have been found to decrease caries experience in these animals. In one of these studies, the establishment of an LDH-deficient mutant in the oral cavity of rats produced a 10-10,000 fold increase in the minimum infective dose necessary for subsequent colonization of the wild-type strain of *S. mutans*.

IMMUNE RESPONSE TO S. MUTANS CELL WALL COMPONENTS AND INDUCTION OF IGA RESPONSES

Results from several NCP supported laboratories indicate that inactivated whole cells of *S. mutans* have immunogens, which can protect against caries. Scientists now are attempting to identify these immunogens and to clarify their effect on cells of the host immune defense system.

The generation of humoral immunity in animals to most antigens requires the cooperative interaction between two types of lymphocytes, the thymus derived (T) and the bone marrow-derived (B) cell. Studies in the neonatal thymectomized (Tx) rat model (depleted of thymus-derived lymphocytes) indicate that T cell deprivation causes a decrease in salivary IgA levels as well as an inability to produce salivary IgA antibodies to a T-dependent antigen (DNP-BGG). Also, local injection of a T-independent antigen (DNP-Ficoll) and *S. mutans* into Tx rats induced significantly less salivary IgA antibody than normal rats. These antibody responses to T-independent antigens may be highly important in protection against dental caries. More recent evidence indicates that decreased salivary IgA responses in Tx rats correlates with an increase in dental caries following *S. mutans* infection. T-cell depleted rats were also shown to exhibit an IgM compensatory reaction in salivary secretions.

It is known that cell walls of Gram positive bacteria possess components which exhibit lymphoproliferative activity and can stimulate cells of the immune system. One of these components, peptidoglycan (PG), is a B cell mitogen, a polyclonal B cell activator for inducing immunoglobulin synthesis, and also serves as an immunopotentiator. Other substances include lipoteichoic acid, which is a T cell mitogen and an adjuvant, and muramyldipeptide (MDP), which acts as an adjuvant. The *S. mutans* cell wall contains all of these substances as well as serotype-specific carbohydrate (CHO).

Recent data indicate that serotype CHO is an effective murine B cell mitogen as well as an excellent polyclonal B cell activator. To determine their immunogenic potential, CHO c and g preparations were coupled to a hapten, trinitrophenyl (TNP). Spleen cell cultures stimulated with these preparations gave good anti-TNP plaque forming cell responses (i.e. measurement of B cell responses to *S. mutans* antigens) and induced significant polyclonal IgM synthesis. Spleen cells from nude mice, lacking a functional thymus, also responded to these preparations indicating that the hapten-CHO conjugates were "T-independent" antigens. Corroboration of the T-independent nature of TNP-CHO was obtained using purified populations of splenic B cells from BALB/c mice, which showed good anti-TNP plaque-forming cell responses.

Several laboratories, supported by NCP grants and contracts, are presently examining purified cell wall components of *S. mutans* for ability to elicit mucosal antibodies and protective immune responses against dental caries in gnotobiotic rats. Investigators have found that gastric intubation of gnotobiotic rats with these components principally induced an IgA response with a concomitant reduction in caries scores. Combining adjuvants (e.g. water/oil/water, liposomes and *S. mutans* PG) with the antigens significantly enhanced both salivary immune responses (S-IgA and IgG) and caries protection. Synthetic adjuvants are now being screened for their ability to potentiate immune responses without untoward reactions.

The adjuvanticity of muramyl dipeptide (MDP), a component of the *S. mutans* cell wall, was also studied. Orally administered MDP enhanced the salivary IgA and IgG response to orally administered *S. mutans* or GTF. The serum IgG response was delayed, but elevated after either oral administration or parenteral injection of MDP. NCP grantees suggest that MDP and its analogs function by directly stimulating macrophages or T helper cells. The primary action seems to be on the macrophage, with liberation of monokines, leading to activation of B cells and T helper cells.

ANTIBACTERIAL SALIVARY FACTORS

Saliva shares with other exocrine gland secretions a number of non-immune antibacterial agents such as lysozyme, lactoperoxidase, lactoferrin and glycoprotein agglutinins. It has been reported that of the plaque proteins assayed in caries susceptible and caries resistant adults, only lysozyme showed a significant concentration difference (i.e. twice as high in the caries resistant group). It is of interest in this regard that scientists have reported that plaque *S. mutans* numbers and caries susceptibility is correlated only in approximal plaque to which there is limited access by saliva.

Studies on cultures of serotype c strains of *S. mutans* indicate that lysozyme causes dechaining and leads to decreased survival in an acidic environment. Fluoride has been reported to sensitize *S. mutans* to killing by lysozyme and to enhance autolytic enzyme activity with consequent cell wall and cell membrane damage. Persons with high caries resistance and receiving fluoride might therefore be expected to have plaques with more dechained *S. mutans*, which would not survive at low pH in the oral environment.

Lysozyme may not be the only salivary component which can give rise to dechaining and altered membrane permeability. For the past three years NCP supported investigators have developed methods to purify and examine the biological properties of human parotid salivary histidine-rich polypeptides (HRP). During growth of *S. mutans* in the presence of HRP, limited dechaining with damage to the cell surface takes place, possibly implicating autolysins. In addition to its growth inhibitory and bactericidal properties, HRP also promotes the aggregation of oral microorganisms.

During the year 83 grants, 6 contracts and 33 direct operations projects were active in Strategy Area I representing 62 percent of National Caries Program Research projects.

STRATEGY AREA II. Increasing the Resistance of the Teeth

Use of fluoride in water supplies, dentifrices, mouthrinses, dietary supplements and in other forms continues to be the most effective way to prevent dental caries. In the U.S. recognition of fluoride's outstanding preventive attributes has come about largely through research, development and promotional efforts of the NCP. Already there is evidence that these efforts are having an effect. Thus findings published in FY 1982 from the NCP-sponsored National Dental Caries Prevalence Survey indicate that the prevalence of dental caries among United States school children decreased 32 percent in approximately the last ten years. The NCP continues to commit a major portion of its resources to evaluating new and more cost-effective ways of using fluorides for caries control.

Evidence accumulates that various combinations of fluoride procedures, particularly those that are believed to act by different mechanisms, produce additive anticaries benefits. To determine the impact of a combination of some of the most feasible procedures, the NCP is studying the longterm anticariogenic effects in children who consume daily a 1 mg fluoride tablet and rinse weekly with a 0.2% NaF solution in school and who receive fluoride dentifrice for home use. Interim results after eight years of the study in Nelson County, Virginia, showed that elementary and junior high school children who had participated continuously in the program had 49% fewer decayed, missing and filled surfaces (DMFS) than their cohorts at the baseline examination. Even more encouraging was the finding that in approximal surfaces, where restorations are the most difficult and costly, dental caries had nearly been eliminated. The program currently is being extended incrementally into the senior high school. Full benefits of the combined regimen will be determined in 1983 when all children through senior high school (grade 12) will have participated in the program since first grade.

School programs of weekly rinsing with a dilute sodium fluoride solution and school programs of daily rinsing with a more dilute sodium fluoride solution have been shown to reduce the incidence of new decay in non-fluoride communities. To determine if one regimen is superior to the other and if fluoride mouthrinsing also confers caries preventive benefits in an optimally fluoridated area, the NCP conducted a study in fluoridated Des Moines, Iowa. Final results after 30 months showed that both regimens effectively prevented dental decay, the daily rinse being slightly, but not statistically superior to the weekly procedure. Because the weekly program takes less school time and effort and costs only one-fourth as much as the daily program, it is recommended as the more cost-effective method.

Currently most of the approximate 10 million school children in the United States that are participating in school-based fluoride mouthrinsing programs reside in non-fluoride areas. The positive findings of the Des Moines trial, plus encouraging results of a few other recent studies, provide a strong basis for promoting the use of fluoride mouthrinsing among the estimated more than 20 million children who live in optimally fluoridated communities.

The fluoridation of a school's water supply at 4 ½ times the concentration considered optimal for community fluoridation in the same geographic area has been shown to reduce the prevalence of dental caries by about 40 percent. Because school water fluoridation and community water fluoridation probably act in similar ways to produce their cariostatic effect, there is reason to believe that additive effects like those obtained in the Des Moines trial can also be achieved by combining fluoride mouthrinsing with school water fluoridation. To confirm this hypothesis, the NCP is planning to implement a four-year study among 1200 children who have consumed fluoridated water at school from the earliest grades. Subjects will be randomly assigned to one of two groups that rinse weekly with either a placebo solution or with a 0.2% NaF solution. This study design will permit early detection of added effects of fluoride rinsing.

Both school water fluoridation and weekly fluoride mouthrinsing in school are feasible, economical, safe, well-accepted by school personnel and students and, once in operation, have minimal need for the direct services of professional dental personnel. If the procedures are shown clinically to impart additive caries-preventive benefits, this knowledge would be important to public health officials establishing programs for caries prevention in areas lacking a central water system.

Findings from an NCP epidemiologic study to help define the current relation between waterborne fluoride concentration and dental fluorosis were presented in 1982 at an international workshop on fluoride. Lifetime residents of four areas of Illinois where public water supplies contained concentrations of natural fluoride varying from optimal (1 ppm) to four times optimal were examined. The data show that the prevalence and severity of fluorosis were distinctly greater at all higher-than-optimal concentrations than at the optimal level. However, a typical dose-response pattern was not found. More importantly, the data show that fluorosis is either similar to or less than that observed by Dean at similar water fluoride concentrations about 45 years

ago. Dental caries experience was also measured in the current survey and it was found that DMFS scores at 2, 3 and 4 times optimal were all significantly lower than at the optimal fluoride concentration.

In reviewing the objectives of the survey, the desirability of having data on dental fluorosis and dental caries from a community with water having only trace amounts of fluoride (a negative control) became apparent. Consequently, in April 1982, children in several neighboring Iowa communities with < 0.3 F in their water were examined. Findings in Iowa will permit a determination of background levels of fluorosis, if any, produced from sources of fluoride other than water and serve to document the continuing efficacy of community water fluoridation.

Numerous animal and human studies have shown that frequent exposure of the teeth to topical fluoride leads to a reduction in dental decay. The NCP has now developed an intraoral device that is designed to provide continual topical fluoride therapy for the prevention of dental caries. The device is a small membrane-controlled, reservoir-type delivery system that can be bonded to the side of a maxillary molar or to an orthodontic appliance. Devices have been fabricated with release rates of 0.02 to 1.0 mg of fluoride per day and durations of action of one to six months. The devices have been subjected to an extensive safety evaluation in animals and the results of these studies indicate that the system should be safe to use in humans.

A device designed to release 0.5 mg of fluoride per day for 30 days has been evaluated in a short-term human test. The 11 men who wore the device had significantly elevated levels of fluoride in their saliva and plaque compared with base line levels. Subsequent caries trials in rats showed that teeth in animals fitted with an fluoride-releasing device developed 54 to 63% fewer carious enamel areas than teeth in rats that received no treatment. In a recently completed trial in monkeys, there were no adverse reactions when animals wore fluoride-releasing devices designed to release 0.2 mg of fluoride per day on their maxillary central incisors for 6 months. A six-month trial in children to document the safety of the device currently is being planned. If no adverse reactions appear in this study the next step will be a three-year large-scale clinical trial to determine caries preventive effectiveness.

The intraoral fluoride-releasing device is being developed to provide caries prevention for very caries-prone individuals. Also, the device should be valuable for mentally or physically handicapped persons who experience difficulty in maintaining good oral hygiene and valuable for persons wearing orthodontic appliances that prevent thorough cleaning of the teeth. Future refinement of the design of the device and the method of retaining it in the mouth could lead to even wider use of this delivery system.

Contract-supported clinical trial activities in FY 1982 included the following:

- Final results were reported from two studies of the effect of stannous fluoride or sodium fluoride mouthrinsing on dental caries, dental plaque, gingivitis, and tooth staining (University of Texas and Eastman Dental Center). Both studies showed that the two agents were similar in their effect on caries and that neither agent produced a lasting effect on plaque or gingivitis after thirty months of supervised use on school days. Slight staining from the use of SnF_2 was observed in both trials.
- Investigators from SUNY at Stony Brook concluded a three-year clinical trial to determine the effect of prior toothcleaning on the efficacy of semi-annual, professionally-applied acidulated phosphofluoride (APF) treatments. Preliminary findings showed that the effect of the gel treatment was not influenced by prior tooth cleaning, whether performed professionally (pumice prophylaxis) or by the subject (supervised brushing and flossing).
- A study has been initiated to determine whether the efficacy of a fluoride dentifrice can be improved by raising the fluoride concentration from 1000 to 2500 ppm. Baseline examinations have been completed. The study will also determine whether a combination of sodium fluoride and sodium monofluorophosphate (MFP) confers greater protection than a formulation of MFP alone.
- An RFP was issued for a clinical study of the effect of dietary fluoride supplements used during pregnancy to prevent dental caries in deciduous teeth of offspring.

The projects highlighted in this section represent a major part of the overall NCP effort in "Increasing the resistance of teeth." During the year, 15 direct operations and 5 contracts were being carried out in Strategy Area II. Including 36 grants in this area, the total coordinated activity amounts to approximately 29% of NCP research projects.

STRATEGY AREA III. Modify the Diet

The importance of dietary factors in the causation of caries is a major concern of the National Caries Program and of dental health professionals working in laboratories and clinics, food manufacturers, and consumers. As a result of this concern 52 percent of households have attempted in the last few years to lessen their intake of sugar by dietary restrictions or by substituting artificial sweeteners.[1] Though the total per capita consumption of sucrose (cane and beet sugar) is declining, the total per capita consumption of fermentable sugars in the United States continues to increase due largely to the increase in use of corn-derived sweeteners (fructose and glucose). A reason for the increase is that sweeteners are widely used in the processed food industry, and occur not only in sweet products, but also as "hidden sugar" in many other products such as certain brands of mustard, table salt, etc. Accompanying the increased per capita consumption of sugar sweeteners in the last 10 years has been a 40 percent increased per capita consumption of the intense sweetener, saccharin, whose use in foods has been extended until August 1983 by a Congressional moratorium on the 1977 FDA ban on saccharine use. Recently after many years of deliberation, the dipeptide ester sweetener aspartame was approved by the FDA for limited use in dry form, such as in table top sweeteners, cereal mixes, packaged drink mixes, and chewing gum. Use in foods containing water such as in low calorie diet sodas has not yet been approved, due to aspartame's significant conversion in water to other products. Also because one of aspartame's metabolic products is phenylalanine, aspartame-containing products must contain a warning for persons with phenylketonurea, who cannot tolerate phenylalanine in their diet. The manufacturer must monitor the amount of aspartame consumed in different products. Aspartame-sweetened products were test marketed in 1982 in several parts of the country, and soon will be widely available.

Because of the wide consumption of products sweetened with cariogenic carbohydrates, the NCP since its inception has sought to develop an array of non-cariogenic sweeteners that potentially can be used in different types of foods and snack items. Currently, research on noncariogenic sweeteners is supported by both grants and contracts. Grantees at the University of California are exploring the molecular parameters necessary to elicit a sweet taste. These scientists and others supported by contract at the Research Triangle Institute have synthesized several intensely sweet dipeptide esters. The stability of these compounds in water relative to that of aspartame is being examined.

A number of plant-derived sweeteners used by native populations in other countries are relatively unknown in the United States. Several of these sweeteners are being investigated through an NCP contract with the University of Illinois, where scientists have isolated, purified, characterized, and subjected to a taste panel all the sweet principles from *Stevia rebaudiana*, a plant widely used in Paraguay. *Stevia* extracts containing several sweet principles with varying taste qualities are used in Japan in commercially-produced foods such as chewing gums, soft drinks, sauces, and pickles. Other sweeteners under study are from the following plants: *Hydrangea thumbergii*, which is native to Japan, *Mormodica grosveneri*, which is native to southern China, and *Lippia dulcis*, which is native to Mexico and southern United States. The work on both synthetic and plant-derived sweeteners is now only at the research stage. After acute toxicity-testing and mutagenicity-testing sweetness qualities are characterized by a taste panel. Subsequently long term chronic toxicity tests are conducted with more than one animal model, in order to assess potential health hazards of the sweeteners. The forthcoming data must then be submitted to the FDA for consideration for approval for use as a sweetener.

In addition to the presence of sugar other factors are known to contribute to or modulate the cariogenicity of foods. These factors include eating frequency, presence of cariostatic agents, food texture, food stickiness, and induction of salivation. To examine these factors NCP scientists have developed a rat model in which essential nutrition commencing at weaning is provided by intubation and in which snack foods are provided at 17 intervals each day. The cariogenic potential index (CPI) determined at the end of 35 days is the ratio of the sulcal caries scores resulting from ingestion of the test food consumed as snacks compared to ingestion of powdered sucrose consumed similarly.

Studies show that the CPI of a food is positively correlated with the ability of the food to support *S. mutans* growth in the oral cavity. Furthermore the CPI of foods of the same type, such as breakfast cereals, tends to increase with increasing sugar content. However, in some cases the measured CPI values do not always follow what conventional wisdom would predict. For example, some potato chips, despite their low sugar content, are highly cariogenic; a caramel candy that was tested, despite its stickiness, is less cariogenic than sucrose itself; and the creme-filled chocolate cookies that were tested are more cariogenic than sucrose. The NCP rat model is being developed as a basic tool with which to elucidate these effects. Because the test food is the only food which comes into contact with the oral cavity, the NCP rat model

also can be used to study effects of snack components on host systems such as salivary enzymes and immunity.

In addition to the rat model, in which caries development is measured, the Program is developing tools for studying plaque acid production in humans. An electrode system capable of measuring pH simultaneously at several points in the oral cavity currently is being tested by NCP scientists. They have found that the pH response varies according to plaque location in the mouth, as well as according to the test food. Through contract with the University of Michigan the NCP also is studying the effects of diet and dietary habits on caries experience in children. The 3-year study will be based on repeated dietary histories, estimation of frequencies of ingestion of various foods, and caries development.

During the year 3 grants, 5 contracts, and 3 direct operations projects were active in Strategy Area III, representing 5 percent of National Caries Program research projects.

(1) 1980 survey by the Economics and Statistics Service, USDA.

STRATEGY AREA IV. Improved Delivery and Acceptance of Caries Preventive Procedures

The operations comprising Strategy Area IV are crucially important to the NCP. These operations utilize educational and promotional materials and activities to transfer caries preventive techniques from the laboratories and clinics where they were developed and tested to the public where they can be put into use. In FY 1982 these operations were markedly curtailed by limited funds and by a Department-wide moratorium on publications and audio-visual materials.

The Program has identified the health professionals who already do play, or could play, key roles in introducing new caries preventive techniques and has continued, with the resources available in 1982, to provide these individuals with information on current research in caries prevention and on benefits and correct use of techniques that are currently available. These key individuals include heads of departments of pedodontics, preventive/community dentistry, operative dentistry and dental hygiene; state dental directors and their supervisory dental hygienists; county and city dental directors; and representatives of various Federal agencies. As described in previous Annual Reports, conferences have been found to be an extremely effective way to communicate information on caries prevention to these in dividuals. A third conference in the series was planned for this year, but was not funded. To conserve expenses, the Program therefore organized and/or supported symposia with similar objectives at annual meetings of national and international organizations. These have been quite successful. The first of these was a symposium titled, "Pit and Fissure Sealants: Is it Time for a New Initiative?" conducted under the auspices of the IADR at its meeting in New Orleans. The objectives of the symposium were: to provide an overview of research on pit and fissure sealants; and to provide a forum for discussion of issues associated with promoting the use of sealants. The session was well attended and the proceedings will be available late this year in the *Journal of Public Health Dentistry*.

In June during the annual meeting of the American Dental Hygienists' Association, the NCP sponsored an all-day symposium, "Dental Caries Prevention: An Update." The conference provided current information on: mechanisms of action, safety and efficacy of fluorides, caries prevention through remineralization, alternative methods of delivering fluorides, status of adhesive sealants, status of new measures to prevent dental caries, and the role of dental hygienists in caries prevention. Because of the value of these topics for all members of the American Dental Hygienists' Association, the Association is publishing the proceedings of the symposium in a special issue of *Dental Hygiene*.

Also, the NCP collaborated with several organizations in presenting the "Minnesota Conference: Dental Caries Prevention in Public Health Programs." This conference, patterned on those presented by the NCP in 1980 and 1981, was sponsored by the Minnesota Department of Health, Hennepin County Dental Program, Minnesota Dental Association, Minnesota Dental Hygienists' Association, University of Minnesota School of Dentistry, and School of Public Health, Program in Dental Public Health. The NCP assisted the organizers in developing the program, and two NCP staff members presented papers and participated in the panel discussion at the conclusion of the two-day conference. The NCP also provided a large assortment of educational materials and an exhibit for use at the meeting. Over 300 participants from Minnesota and neighboring states attended, including dentists, dental hygienists, school administrators and nurses, public health educators, nurses and physicians, representatives from the women's dental auxiliary, Head Start administrators and health coordinators, representatives of dental insurance carriers, and the dental materials industry.

During 1982, the Program sent to all 201 schools or departments of dental hygiene a letter to review the purpose of the NCP and to advise them of the availability of NCP's educational materials for use in teaching dental hygiene students. The response to this offer has been excellent, and requests for materials continue to be received as the new academic year begins.

In FY 1982 the Program was able to publish a revised and expanded version of *Preventing Tooth Decay: A Guide for Implementing Self-Applied Fluorides in School Settings*. On the other hand, as mentioned earlier, the Program's efforts to develop new educational aids for use by health professionals and the general public were critically impacted by the Department-wide moratorium on educational materials. Two posters had been planned, one demonstrating the combined benefits of fluorides and fissure sealants and the other recommending the use of fluorides by adults. Production of both of these badly needed posters has had to be postponed. Also the reprinting of two, highly valuable leaflets, "Fluoride Mouthrinsing in Schools... Protection for Children's Teeth" and "A Healthy Start...Fluoride Tablets for Children in Preschool Programs," was considerably delayed in the request, review and appeal process. Finally, even though the decision was appealed twice, the NCP did not receive permission to produce much-needed films and T.V.

spots that are designed to educate the public about fluorides.

As shown in Table I, the four films produced by the NCP in 1979 and made available on free-loan in 1980 are still well used.

In FY 1982, the NCP distributed over 388,756 publications emanating from 10,789 individual requests, as shown in Table II. These figures do not represent materials distributed by NCP staff at meetings where the large exhibit was displayed.

In FY 1982, NCP's major scientific exhibit on school-based self-applied fluoride programs was staffed by NCP personnel for a total of 26 days at the annual sessions of the following organizations: American School Health Association, American Public Health Association, National Association of Pediatric Nurse Associates and Practitioners, National School Boards' Association, American Dental Hygienists' Association, and the American Nurses' Association. Again, this year NCP staff presented papers on caries prevention during the scientific sessions of several of these meetings.

Other NCP exhibits were staffed by NCP personnel at seven meetings, including the National Association of Black School Educators, the New Jersey League for Nursing, and the American Federation of Teachers for a total of 15 days.

Eleven new free-loan table-top exhibits on the use of self-applied fluorides in schools became available in January. These exhibits were displayed at 62 sites including health fairs, continuing education courses, parent-teacher meetings, in service workshops, and at dental, dental hygiene, and public health association meetings. Persons who request a free-loan exhibit usually request and distribute educational materials prepared by NCP, as well.

Staff members continue to provide consultation to local, state, and national groups in connection with implementing and monitoring supervised, self-applied fluoride regimens. In addition, they give continuing education courses on caries prevention to a variety of health professionals, lecture on caries prevention to dental and dental hygiene students, and organize and participate in symposia and programs at many professional meetings.

In addition to the activities described above in the Office of the Associate Director, 3 grants, 2 contracts and 1 direct operations project were active in FY 1982 in Strategy Area IV. The latter projects represent about 4 percent of NCP's total grant, contract and direct operations projects.

Table I

Title of Film	Months in Circulation	Bookings	Showings	Viewers
The Daily Tablet for Healthier Smiles	33	346	504	11,706
The .2% Solution	34	577	1,169	46,377
Smilemakers: Self-Applied Fluoride Programs for Schools	34	613	913	25,849
Prescribing Fluoride Supplements in Medical and Dental Practice	31	870	1,111	20,461
Totals for 4 Pictures		2,380	3,629	104,393

Table II

TITLE OF PUBLICATION	NUMBER DISTRIBUTED
Preventing Tooth Decay: A Guide for Implementing Self-Applied Fluorides in School Settings	2,720
Dental Caries Prevention in Public Health Programs - Proceedings of a Conference	1,794
Reprints (Results of studies and reviews written by NCP staff about community based self-applied fluoride regimens, professionally applied fluorides, and prescribing dietary fluoride supplements)	810
Leaflets	
Fluoride Mouthrinsing in Schools... Protection for Children's Teeth	218,397
Fluoride Tablets: A Healthier Smile for School Children	23,093
A Healthy Start...Fluoride Tablets* for Children in Preschool Programs	25,031
Fluoride to Protect Your Children's* Teeth	18,015
Oral Health Education and Promotion Materials from the National Caries Program	4,696
(Film leaflets with order card) You can be a moving force for better oral health in your community	3,762
Prescribing Fluoride Supplements in Medical and Dental Practice	4,050

*The low number of leaflets distributed reflects a long period of low supply.

Subtotal -	302,368
Posters	86,388
Grand Total -	388,756

NATIONAL CARIES PROGRAM

Notice of Intramural Research Project Forms

Fiscal Year 1982

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 OE 00029-15 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982 — CT-0600057

TITLE OF PROJECT (80 characters or less)

The effect of school water fluoridation on dental caries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Heifetz, Stanley B.	Clinical Investigator	NCP CPR NIOR
Horowitz, Nerschel S.	Chief, CP Section	NCP CPR NIOR
Brunelle, Janet A.	Chief, B Section	NCP CPR NIOR
Meyers, Rhea J.	Clinical Investigator	NCP CPR NIOR

COOPERATING UNITS (if any)

Dental Health Division, North Carolina State Board of Health, Division of Water Hygiene, Environmental Protection Agency

LAB/BRANCH: Caries Prevention and Research

SECTION: Community Programs

INSTITUTE AND LOCATION: NIOR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fluorides were added to the water supply of a school in Seagrove, North Carolina. The concentration of fluoride used was 7 times higher than the level considered optimal for community water fluoridation in the geographic area. Children attending the Seagrove school live in an area where the various sources of well water contain negligible levels of fluoride. Baseline dental examinations for dental caries were made prior to the installation of fluoridation equipment. Follow-up examinations were conducted after four, eight, and twelve years to determine the extent of caries protection as increasingly larger segments of the study population become continuously exposed to fluoridated water at school since entering the first grade. Results of the four- and eight-year examinations on full beneficiaries of the procedure showed decreases in caries prevalence of 30 and 40%, respectively, compared with baseline findings. On the eight-year examinations, an assessment of the prevalence of dental fluorosis showed that no children had any definite signs of the condition.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00032-14 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982 — CT 0060042

TITLE OF PROJECT (80 characters or less)

Effects of chewable fluoride tablets on dental caries in school children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Driscoll, William S.	Clinical Investigator	NCP CPR NIOR
Heifetz, Stanley B.	Clinical Investigator	NCP CPR NIOR
Brunelle, Janet A.	Chief, B Section	NCP CPR NIOR

COOPERATING UNITS (if any)

Wayne County Public School System, North Carolina

LAB/BRANCH: Caries Prevention and Research

SECTION: Community Programs

INSTITUTE AND LOCATION: NIOR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study was initiated in October 1969 with 1034 children in the first and second grades of nine schools located in Wayne County, North Carolina, an area that has negligible amounts of fluoride (F) in its supplies of drinking water. Following baseline dental examinations, in which the DMF surface index was used, the children were stratified according to certain variables and then randomly assigned to one of the following three study groups: Group A (controls) chewed a placebo tablet, rinsed their teeth for 30 seconds with the resulting salivary solution, and then swallowed the material; Group B followed an identical procedure using an acidulated phosphate-fluoride (APF) tablet that contained 1 mg. F; Group C followed the same procedure as Group B except that, after at least 3 hours, the procedure was repeated with a second APF tablet that also contained 1 mg. F. The procedures were carried out each day in school under the classroom teacher's supervision for a period of six years. Interim follow-up examinations were conducted in April 1972, May 1974, September 1975 and May 1977. Final examinations were conducted in May 1979.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 OE 00070-10 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982 — CT 050004S

TITLE OF PROJECT (80 characters or less)

Combined self-applied fluorides for caries prevention in a non-fluoridated area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Horowitz, Herschel S.	Chief, CP Section	NCP CPR NIOR
Heifetz, Stanley B.	Clinical Investigator	NCP CPR NIOR
Meyers, Rhea J.	Clinical Investigator	NCP CPR NIOR
Oriscoll, William S.	Clinical Investigator	NCP CPR NIOR
Li, Shou-Hua	Statistician (vis.)	NCP CPR NIOR

COOPERATING UNITS (if any)

Nelson County, Virginia, Public School System

LAB/BRANCH: Caries Prevention and Research

SECTION: Community Programs

INSTITUTE AND LOCATION: NIOR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Baseline dental examinations were conducted in October 1972, on approximately 2200 children (grades 1-12). All participants in grades K-6 chew daily in school under supervision a sodium fluoride tablet containing 1 mg. F, and swish and swallow the resultant solution. Once a week in school the children also swish a 0.2 percent sodium fluoride solution. On a scheduled basis, a fluoride-containing dentifrice and toothbrushes are distributed to the children for use at home. Kindergarten classes were invited to participate in the program beginning in the 1976-77 school year. Children in the 7th and 8th grades in Nelson County's junior high school began to participate in the program in the fall of 1978 and 1979, respectively. Beginning in the fall of 1980, high school students in Nelson County began to participate only in the tablet and dentifrice components of the program. For the period covered by this report, children in grades K-10 were participating.

PHS-6040 (Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 OE 00112 09 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Preclinical screening of anticaries agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR
Kingman, Albert	Statistician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

American Dental Association Health Foundation, National Bureau of Standards, Gaithersburg, MD. Drs. W.E. Brown and L.C. Chow

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal objectives of this project are part of the on-going effort identifying antiplaque and anticaries agents suitable for short-term clinical investigation, as well as, to develop methods for assessing the clinical potential for these agents; e.g., its staining properties. Octenidine has been identified by our laboratory as an agent which restricts dental plaque and caries in rats. Used as a rinse twice daily in a 1% solution, the results compared favorably to chlorhexidine (Annual Report 1980-1981). In this study an oral rinse of 1% octenidine was found to provide caries restriction when used only once daily. Our efforts also have been directed toward developing a rat staining model as a means of improving the drug screening process.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00113 09 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Short-term clinical trials of antiplaque and anticaries agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR
Brunelle, Janet A.	Chief B, Section	NCP CPR NIDR
Bowen, William N.	Chief, CPR Branch	NCP CPR NIDR
Kennedy, John B.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)
Department of Periodontology, School of Dentistry, U. of PA, Philadelphia, PA
Drs. S.L. Vankell, P.A. Green and N. Stoller

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) BLOODS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are: (1) to identify, adapt and pretest methods of measuring the bacterial and chemical composition of dental plaque and saliva and (2) to conduct short-term clinical studies of agents which might be capable of restricting dental plaque and caries. The present pilot studies investigated the time course of fluoride following various types of fluoride treatment. The first study investigated the pharmacokinetics of a fluoride releasing device immediately after attachment. Fluoride levels increased were markedly elevated in saliva within an hour of attachment. However, much of the fluoride remained near the point of device attachment. The second study investigated the effect of two gel regimens on oral and systemic fluoride levels. These two regimens were similar except that in one regimen, the treatment was followed by thorough oral rinsing with water. This pilot evaluation indicates that the no-rinse procedure (unlike the rinse procedure) markedly elevates the fluoride level in the serum and urine. Both gel regimens provide markedly increased fluoride levels in the saliva.

PHS-6043
(Rev. 3-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00147-08 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Lectins in the study of plaque and caries development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) BLOODS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lectins, which are proteins capable of interacting with certain macromolecules and/or cell types via specific sugar moieties, are being used to investigate the interactions between saliva and/or bacteria in order to better elucidate the role these interactions play in plaque and caries development. Findings to date support the conclusion that 4 lectins, wheat germ agglutinin, Concanavalin A, fucose binding protein and soybean agglutinin, can reversibly bind to and inactivate by complexation and/or precipitation the aggregating factor in saliva that is responsible for inducing the aggregation of Streptococcus mutans cells. These results provide evidence that the salivary aggregating factor contains N-acetyl-D-glucosamine (GlcNAc), D-mannose and/or D-glucose, L-fucose and N-acetylgalactosamine (GalNAc) and/or D-galactose (D-Gal). Lectins specific for D-Gal, D-GlcNAc, and D-GalNAc have been the most effective for the direct aggregation of oral bacteria.

PHS-6043
(Rev. 3-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00154 08 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biochemical product and energy requirements of plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Robrish, Stanley A.	Research Scientist	NCP CPR NIDR
Kemp, Christopher W.	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Sharer, Sue A.	Laboratory Assistant	NCP CPR NIDR
Curtis, Michael A.	Laboratory Scientist (vis.)	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD

TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) BLOODS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Plaque fluids obtained from monkeys before ad after application of sucrose have been analysed for their volatile metabolic products using high resolution capillary column and a gas chromatograph. A variety of volatile fatty acids was demonstrated in the plaque fluid samples and a decrease in the proportions of acatic and propionic to butyric acids was demonstrated following application of sucrose seen.

Plaque fluid samples obtained from irradiated and control monkeys have been analysed for L (+) and D (-) lactic acid. Following sucrose application, there was an expected sharp rise in the amount of the L (+) lactate. High concentrations of the D (-) form was found in all of the samples.

Methane formation has been demonstrated in dental plaque and the reduction of proline to delto amino valeric acid (DAVA) determined. A pure culture of one of the organisms responsible for DAVA formation has been isolated.

PHS-6043

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00190 07 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Bacterial extracellular macromolecules and colonization of oral bacteria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Rolla, Gunnar R.	Laboratory Scientist (vis.)	NCP CPR NIDR
Futakami, Katsuyuki	Guest Worker	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Merad, Steven A.	Costep (Dental)	NCP CPR NIDR
Steckowych, Lee N.	Student Volunteer	NCP CPR NIDR
Forquer, Kelly A.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)
National Institute of Allergy and Infectious Diseases, Dr. Theodore Theodore
University of Goteborg, Swedan - Dr. Jan Olsson

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) BLOODS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our earlier experimental results suggested a role for lipoteichoic acid (LTA) in the colonization of oral streptococci on mucosal surfaces. Continuing studies have shown that LTA can influence the adsorption of certain strains of S. salivarius, S. sanguis and S. mutans to human and/or monkey buccal mucosal cells. The results indicate a role for this amphiphile in the adherence of oral bacteria to mucosal cells similar to that reported for group A streptococci.

Bacterial glucosyltransferase (GTF), fructosyltransferase (FTF), dextranase and lipoteichoic acid (LTA) were found in monkey dental plaque and/or in human saliva. A relationship between levels of these bacterial metabolites and levels of dental caries remains to be determined. Although salivas from some humans with low GTF/FTF activities also had low levels of total streptococci and/or S. mutans, there was no relationship, in general, between amounts of bacteria and enzyme activities or LTA.

PHS-6043
(Rev. 3-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 201 DE 00206-06 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982 CT-060011B

TITLE OF PROJECT (80 characters or less)

Effect of daily and weekly rinsing with sodium fluoride solutions in a non-fluoridated area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Heifetz, Stanley B.	Clinical Investigator	NCP CPR NIDR
Meyers, Rhea J.	Clinical Investigator	NCP CPR NIDR
Kingman, Albert	Statistician	NCP CPR NIDR

COOPERATING UNITS (if any)

Biddeford School Department, Biddeford, Maine

LAB/BRANCH: Caries Prevention and Research

SECTION: Community Programs

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In 1976, a sodium fluoride (NaF) mouthrinse study was started in Biddeford, Maine, a non-fluoride area. Baseline dental examinations (DMFS Index) were made of 825 children in grades 5-7 attending seven schools in the community. Participants were randomly divided into three groups. Under teacher supervision, they rinsed either weekly with a 0.2% NaF solution or a 0.1% sodium chloride solution (Placebo) or daily with a 0.05% NaF solution. Treatments were carried out for three school years. Follow-up dental examinations were scheduled annually to compare the anti-caries effectiveness of the two fluoride mouthrinse procedures. The third and last year of treatments and final examinations were carried out in 1979.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 201 DE 00220-06 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982 CT 0060121

TITLE OF PROJECT (80 characters or less)

Comparison of daily and weekly rinsing with sodium fluoride in a fluoridated community.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Driscoll, William S.	Clinical Investigator	NCP CPR NIDR
Swango, Philip A.	Project Scientist	NCP CRGC NIDR
Horowitz, Alice M.	Public Health Educator	NCP OAD NIDR
Kingman, Albert	Statistician	NCP CPR NIDR

COOPERATING UNITS (if any)

Des Moines Independant Community School District, Iowa

LAB/BRANCH: Caries Prevention and Research

SECTION: Community Programs

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study was initiated in September 1977 with 1000 children in the seventh grade of nine junior high schools located in Des Moines, Iowa, a community that has optimal amounts of fluoride in its supply of drinking water. The children were randomly assigned to one of the following three study groups: Group I (controls) rinsed their mouths once every week in school for 60 seconds with a placebo solution; Group II followed an identical procedura using a 0.2% neutral sodium fluoride solution (0.09%F). Group III rinsed their mouths once every day in school for 60 seconds using a 0.05% neutral sodium fluoride solution (0.023%F). The procedures were carried out under the classroom teacher's supervision for a period of three years. Baseline dental examinations, using the DMF surface index, were conducted in November 1977. An interim, follow-up examination was conducted in April 1979 and the final examination was conducted in May 1980.

PHS-6040 (Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00222-06 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Specific and non-specific immune factors in plaque fluid and saliva

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Hsu, Su-Cheng O.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

National Institute on Aging - Dr. B. Baum

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The free aqueous phase was obtained from individual samples of dental plaque and the plaque matrix was then eluted with a chaotropic buffer in an attempt to remove bound protein. The fluid and the chaotropic phases were assayed for secretory immunoglobulin A (SIgA), IgG, IgM, the third component of complement (C'3), lysozyme, lactoperoxidase and lactoferrin. The presence of these specific and non-specific immune factors in the free and bound phases suggest they are important in host defense at the plaque-enamel interface. Analyses by S.O.S. PAGE and immunoblotting revealed that many of the immune protens were degraded into small molecular weight fragments.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00225 06 CPR

PERIOD COVERED: October 1, 1981 to September 31, 1982

TITLE OF PROJECT (80 characters or less)

Cost analysis of implementing school-based community mouthrinse programs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Brunelle, J.A.	Chief, B Section	NCP CPR NIDR
Miller, A.J.	Project Scientist	NCP CPR NIDR
Doherty, N.	Economist	Uni. of Connecticut

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention Branch

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data on costs of administering the program, student participation and caries experience were collected from seventeen communities throughout the U.S. and Guam which conducted three-year demonstrations of school-based mouthrinse programs in grades K-6/8. An additional three years of data was collected from five programs which later extended the regimen into the Junior and Senior High Schools. Analysis of implementation costs, acceptance of community and cost-effectiveness were performed during and after the five year period.

PHS-6040 (Rev. 8-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00229 06 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Plaque variations in populations ingesting different levels of water fluoride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Stiles, Horace M.	Chief, P Section	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Brunelle, Janet A.	Chief, B Section	NCP CRP NIDR
Dinsmore, Edwin E.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three communities having 4.2, 2.3 and less than 0.1 ppm fluoride in the drinking water were chosen as study sites. Children 12-18 years of age who had been lifelong residents in the communities comprised the study populations. Plaque and saliva samples, collected from each participant, have been analyzed for and fluoride content. DMF surfaces also were recorded for each participant. Data consists of comparisons of various parameters among the three groups.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00234 05 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Develop method of intraoral telemetry of various ions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)
Microelectrodes, Londonderry, NH Dr. Normand Hebert

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our laboratory is developing several types of oral telemetry which enable direct and continuous measurements of H^+ and F^- levels following ingestion of various foods and therapeutics. A newly developed wire telemetry device has been developed which allows measurement of five interdental areas simultaneously. This design permits reproducible positioning of the pH sensors when assessing the effects of various foods on the pH levels in dental plaque. The pH responses detected following ingestion of various foods produced pH curves similar to those reported by other investigators. The responses vary within the mouth of a given individual. Data from telemetry coordinated with data from other projects will aid in the evaluation of various food stuffs for their relative cariogenicity.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00243 05 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Growth, energetics, and interaction of plaque microorganisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Kemp, Christopher W.	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Sharer, Sue A.	Laboratory Assistant	NCP CPR NIDR
Curtis, Michael A.	Laboratory Scientist (vis.)	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A Streptococcus sanguis and S. mutans were grown in the continuous mode both singly and in coculture. The results showed that S. sanguis had a higher affinity for glucose used as a limiting energy source than S. mutans, however, S. mutans appeared to produce an inhibitor to S. sanguis. Amino acid analysis of the culture medium following growth of these two organisms has helped to explain a discrepancy between the constant molar growth yields observed and the different fermentation products formed at high and low dilution rates.

The growth parameters of some S. mutans isolates, with altered caries potential and altered growth on sucrose, have been calculated from continuous cultures. Polysaccharide fractions have been isolated from the parent and variant of an S. mutans strain altered in its colonial morphology on sucrose. The structure and composition of these polymers is now under investigation.

Lactobacillus casei and S. sanguis have been grown in complex media on limiting glucose without pH control and have lowered the pH of the medium while maintaining high cell densities.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00262 04 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of an intraoral device designed for providing sustained low levels of fluoride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR
Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Kingman, Albert	Statistician	NCP CPR NIDR
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR
Li, Shou-Hua	Statistician (vis.)	NCP CPR NIDR
Little, Wayne A.	Laboratory Technician	NCP CPR NIDR
Kemp, Christopher W.	Laboratory Technician	NCP CPR NIDR
Kennedy, John B.	Laboratory Technician	NCP CPR NIDR
Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR
Sharer, Sue A.	Laboratory Assistant	NCP CPR NIDR

COOPERATING UNITS (if any)
NCI, NIH - Elizabeth W. Chu NCI, NIH - Luz Gelito
Southern Research Institute (SRI), Birmingham, Alabama - Dr. D.R. Cowsar
Hazelton Laboratories, Oral Research Section, Vienna, VA. - Dr. D. Dalgard

LAB/BRANCH
Caries Prevention and Research Branch

SECTION
Preventive Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation supplements earlier studies done by Dr. Mirth and coworkers to develop a fluoride releasing device (FRD) for intra-oral use. This investigation, which is still in progress, is assessing the safety and efficacy in eight monkeys (Macaca fascicularis) of a FRD designed to release 0.2 mg F daily for six months. Inspection of the data sugggests that there has been marked elevation of fluoride levels in the dental plaque and saliva but not in the serum. No untoward local or systemic effects were observed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00263 04 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cariogenicity of the different serotypes of S. mutans in gnotobiotic rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Thomson, Lynn A., Jr.	Laboratory Scientist	NCP CPR NIDR
Little, Wayne A.	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

Gnotobiotics Unit, DRS, NIH

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of gnotobiotic experiments is being conducted to determine the relative cariogenicity of different serotypes of S. mutans and certain S. mutans isolates of interest. These monoinfected rat experiments follow a stringent standardized test regimen. Experiments are conducted sequentially as only one or two isolators are avilable at a time and breeding facilities have limited the availability of 18-20 day old Osborne-Mendel rats. Replacement of the previous breeding diet has resulted in a significant increase in liter size. Results to date suggest that significant differences do exist in the cariogenicity of different strains. Careful analysis of caries scores has prompted the shortening of the experimental periods from eight to six weeks. Improved methods to insure a uniform innoculum from archived strains of S. mutans have been adopted. Additional experiments are currently being conducted to determine the parameters of these differences in cariogenicity and the reproducibility of the standardized test conditions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00274 04 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Host proteins and colonization of oral streptococci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Rolla, Gunnar R.	Laboratory Scientist (vis.)	NCP CPR NIDR
Afseth, John	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Forquer, Kelly A.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

Univeristy of Goteborg, Sweden - Dr. Claes-Goran Emilson and Dr. Jan Olsson

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology Section

INSTITUTE AND LOCATION: NIH, NIDR, Bethesda, MD 20205

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our previous findings suggested that high and low levels of infection in humans by certain strains of S. mutans may be related to the ability of saliva to induce bacterial aggregation. Such a relationship was not observed with humans with intermediate levels of infection. Further studies with more human volunteers showed a diurnal variation in saliva-induced aggregation between 10 and 30%. In one isolated case as much as a 70% variation was observed. The ability of saliva to aggregate oral streptococci appeared not to be related to the numbers of indigenous streptococci or S. mutans present in the saliva.

Results of recent experiments showed that host-produced IgA, IgG, albumin, lysozyme and two thermo-stable, acidic proteins were present in human saliva adsorbed to hydroxyapatite. Preliminary results showed that preparations of IgG, IgA, and thermo-stable protein stimulated glucan synthesis by S. mutans glucosyltransferase; lysozme reduced glucan formation. The effects of these saliva-associated host proteins on bacterial aggregation, adsorption of bacteria to hydroxyapatite and on glucosyltransferase-mediated aggregation and adherence of oral streptococci are under investigation.

FS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00277-03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Prevalence of dental caries and dental fluorosis in areas with optimal and above optimal concentrations of fluoride in their water supplies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Horowitz, Herschel S.	Chief, CP Section	NCP CPR NIDR
Heifetz, Stanley B.	Clinical Investigator	NCP CPR NIDR
Meyers, Rhea J.	Clinical Investigator	NCP CPR NIDR
Driscoll, William S.	Clinical Investigator	NCP CPR NIDR
Kingman, Albert	Statistician	NCP CPR NIDR

COOPERATING UNITS (if any)

Division of Dental Health, Illinois Department of Dental Health, and Eugene R. Zimmerman, Baylor College of Dentistry

LAB/BRANCH: Caries Prevention and Research

SECTION: Community Programs

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In 1980, a cross-sectional survey to measure the prevalence of dental caries and dental fluorosis was conducted in several study sites served by public water supplies that contained natural fluorides at approximately the optimum concentration recommended for maximal caries protection, and at two, three and four times the optimum. Only children who were continuous residents since birth at each site and who used the public water supply as their primary source of drinking water were included in the survey. About 800 children, ages 8-15 years, were examined. Dental caries was assessed with the DMF surface index and dental fluorosis was measured traditionally with Dean's Index and with a newly developed Tooth Surface Index of Fluorosis (TSIF). Fluorosis was assessed independently in each child by each Index. In addition, color photographs of the teeth of some children were taken to depict varying degrees of fluorosis. In the spring of 1982, a group of children from four communities in Iowa with negligible concentrations of fluoride in their drinking water were examined for comparison with the children in Illinois.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00278 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Induction of secretory immunity against Streptococcus mutans in human subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR
Li, Shou-Hua	Statistician (vis.)	NCP CPR NIDR

COOPERATING UNITS (if any)

Dept. Cariology, U. Goteborg, Goteborg, Sweden - Dr. Claes-Goran Emilson

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Baseline levels of antibody in whole and parotid saliva and serum reactive with S. mutans were determined in eight human volunteers. The volunteers were then infected with S. mutans strains Ingbritt (serotype c) and OMZ 65 (serotype d/g) which were resistant to streptomycin. After both serotypes were shed from the mouth, the subjects were immunized against OMZ 65 by swallowing 25mg of formalin killed bacteria in capsules for three successive days and the subjects were reinfected with strains Ingbritt and OMZ 65. After the bacteria were again shed the immunization and implantation cycle was repeated except that capsules were ingested on seven successive days. Samples of blood, whole and parotid saliva and tears were collected throughout the experiment and assayed for antibodies to OMZ 65 and Ingbritt by enzyme and fluorescein linked immunoabsorbant assays. Antibody in the IgA class reactive with OMZ 65 and Ingbritt was induced in whole and parotid saliva by immunization. The presence of antibody was accompanied by reduced implantation and colonization of OMZ 65.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00279 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of secretory immunity Against S. mutans on its colonization in human subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR
Li, Shou-Hua	Statistician (vis.)	NCP CPR NIDR

COOPERATING UNITS (if any)

Dept. of Cariology, University of Goteborg, Goteborg, Sweden - Dr. Claes-Goron Emilson

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eight human volunteers were infected with S. mutans strains Ingbritt (serotype c) and OMZ 65 (serotype d/g) which were resistant to streptomycin; the level of implantation and duration of colonization were monitored. After both serotypes were shed from the mouth, the subjects were immunized against OMZ 65 by swallowing 25mg of formalin killed bacteria in capsules for three successive days and the subjects were reinfected with strains Ingbritt and OMZ 65. The level of implantation and duration of colonization were again monitored. After the bacteria were shed the immunization and implantation cycle was repeated except that capsules were ingested on seven successive days. Antibody in the IgA class reactive with OMZ 65 was induced in whole and parotid saliva by immunization. The presence of antibody was accompanied by reduced implantation and colonization.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00281 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Analysis of oral fluids using high performance liquid chromatography (HPLC)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Adderly, Donne D.	Laboratory Tecnician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recent advances in high performance liquid chromatography (HPLC) column technology have made it possible to rapidly anelyze protein samples using HPLC. The present study has shown that NPLC can be used to monitor protein purification schemes, to analyze commercial proteins such as secretory - IgA (S-IgA) and lactoferrin for purity, and to obtain comparative protein profiles from saliva and plaque fluid. Preliminary results suggest that HPLC can also be used to quantitate constituents of salive such as S-IgA and to rapidly isolate S-IgA from saliva.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00282 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Anticaries evaluation of an intraoral fluoride-releasing device in rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR
Amsbaugh, Suzanne M.	Laboratory Technician	NCP CPR NIDR
Song, Lucinda J.	Laboratory Technician	NCP CPR NIDR
Li, Shou-Hua	Statistician (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous in vitro and in vivo studies have shown that an intraoral fluoride releasing device developed by the Southern Research Institute for the National Caries Program will deliver fluoride at a steady rate for up to six months. This project will evaluate the anticaries effect and mechanism of action of the fluoride-releasing device in rats. Results have shown that rats that had an intraoral fluoride releasing device in their mouth developed significantly fewer caries on all surfaces than untreated or placebo treated animals. The data also indicate that the marked caries reduction produced by the fluoride releasing device was due to topical effects of fluoride.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00292 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Analysis of the National Dental Caries Prevalence Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Brunelle, J.A.	Chief, B Section	NCP CPR NIDR
Miller, A.J.	Project Scientist	NCP CPR NIDR
Smith, J.	Statistician	NCP CPR NIDR
Rodgers, P.A.	Statistical Assistant	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A nationwide survey to assess the prevalence of dental caries throughout the United States was designed and implemented during the 1979-1980 school year. A probability sample of school-aged children in grades kindergarten through twelve was selected for examination for dental caries, gingivitis, and need for dental treatment. Estimates of disease level and treatment need were made for the continental U.S. and each of seven geographic regions by age, race and sex.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00294 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (60 characters or less)
An approach to analyzing treatment effects in a clinical trial using Markov chain methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kingman, A.	Statistician	NCP CPR NIDR

COOPERATING UNITS (If any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Assessing the severity of dental caries in subjects has been attempted by several researchers. The usual method is the use of the DMFS index. This index counts the number of decayed, missing or filled surfaces detected in the subject. This index has been of limited value in terms of its ability to predict the levels of new caries activity expected in specific subjects. Another approach to classifying severity was to partition the subjects' dentition into distinct regions and record the presence or absence of decay in each region. The MGSI index assigned to the subject a score representing the number of regions (based on those defined by Grainger) in which evidence of caries was detected. This resulted in every subject being assigned a value from 0 through 5. It was shown in an earlier study by Kingman that this method of assigning severity of dental caries was a better predictor of future caries activity than the DMFS index.

Therefore, the current investigation is being undertaken to attempt to evaluate treatment effects in a longitudinal clinical trial and compare these results with those one would obtain by using an analytical method based on the DMFS index itself.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00295 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (60 characters or less)
Effect of fluoride pulse on Streptococcus mutans in continuous culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kemp, Christopher W.	Laboratory Technician	NCP CPR NIDR
Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Sharer, Sue A.	Laboratory Assistant	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (If any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A pure culture of S. mutans at steady state in a continuous culture (chemostat) was pulsed with a mixture containing sodium fluoride and ^{14}C labeled glucose. The pulse was applied to steady state populations at a low and high growth rate for pH values of 7.0, 6.2 and 5.4. Samples were obtained before the pulse and at timed intervals after the pulse. The samples have been analyzed for fluoride, residual glucose, ^{14}C label incorporation, dry weight, ATP, lactic acid and volatile fermentation products.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00296-03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (60 characters or less)
Effect of immunization with Actinomyces viscosus T-6 on colonization in the rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (If any)
Department Cariology, University Goteborg, Goteborg, Sweden - Dr. Jan Olsson

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Four groups of noninfected rats, 12 in each group, were injected either intraperitoneally or in salivary glands with either formalin killed Actinomyces viscosus plus Freunds adjuvant or Freunds adjuvant alone. The procedure was repeated twice at 14 day intervals. The animals were then grouped so that each cage contained one immunized animal, one sham immunized animal and a third animal which had previously been infected with Actinomyces viscosus (T-6). The rate of implantation in the non-infected animals was monitored and the level of antibodies against T-6 in serum and saliva was determined. Both salivary gland (SG) and intraperitoneal (IP) immunization resulted in high levels of SIgA antibody in saliva and IgG antibody in serum. Low levels of IgG antibody in saliva and IgM antibody in serum were also induced. Intraperitoneal immunization was approximately twice as effective as SG immunization. The level of implantation was inversely related to the level of salivary and serum antibody.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00298-03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (60 characters or less)
Effect on cariogenicity and saliva composition of a suboptimal diet in rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR

COOPERATING UNITS (If any)
Dept. of Cariology and Laboratory of Biochemistry, Univ. of Umea, Sweden - Dr. Thorild Ericson; Dr. Ingegerd Johansson.

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Some properties of saliva such as buffer capacity, secretion rate, anti-bacterial activities and effects on bacterial colonization are suggested to be of importance for the development of dental caries. Such properties would be dependant upon the biosynthesis and secretion of substances from the salivary glands. The aim of this study is to investigate the effect of malnutrition on the composition of whole saliva and on incidence of caries in rats fed sucrose. Twenty rats were fed nutritionally adequate diets by gastric intubation. Ten of these received a supplement of sucrose and 10 of starch. Another 20 rats were fed the basic diet diluted with an equal volume of water; ten were supplemented with sucrose and with starch. The supplements are distributed 17 times daily. Striking difference in caries score in the two sucrose fed groups (6.5 - 35.0). No differences were evident in the activity of lactoperoxidase or lysozyme.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DE 00304 03 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Production of Strep. mutans serotype c antisera

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Little, Wayne A.	Laboratory Technician	NCP CPR NIDR
Thomson, Lynn A. Jr.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigation into methods for improving antisera production against a variety of oral bacteria has been an area of continued research in our laboratory. Streptococcus mutans serotype c has been of particular interest because of its prevalence in humans and, in general, poor immunogenicity in rabbits. Previous studies have examined different variables including immunization schedule, vaccine strain and vaccine dose in an attempt to produce the high titered antisera necessary for reagent grade fluorescent antibody conjugates. More recently, we have investigated the effect of slow, continual release of antigen into rabbits through use of Alzet® minipumps.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DE 00310-0Z

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Evaluation of fluoride mouthrinsing and fluoride tablets when used separately and in combination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Horowitz, Herschel S.	Chief, CP Section	NCP CPR NIDR
Meyers, Rhea J.	Clinical Investigator	NCP CPR NIDR
Heifetz, Stanley B.	Clinical Investigator	NCP CPR NIDR

COOPERATING UNITS (if any)

Springfield, Ohio, Public and Non-Public Schools

LAB/BRANCH: Caries Prevention and Research

SECTION: Community Programs

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Approximately 1800 kindergarten and first grade children were randomly assigned to one of the following groups:

Group I - Rinses once every week in school with a 0.2% sodium fluoride solution.

Group III - Ingests once a day in school a sodium fluoride tablet containing 1 mg. of fluoride.

Group II - Carries out the regimens for both Group I and Group III.

The method of assignment resulted in three comparable groups each containing about 600 children. Participants carry out their assigned treatments in classrooms under the close supervision of a teacher. Treatments will be administered for a minimum of eight and nine years for first grade and kindergarten, respectively. Baseline dental examinations were conducted in September 1981. The prescribed treatments were initiated shortly after the examinations were completed. The children have completed one year of their respective treatments.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DE 00319 02 CPR

PERIOD COVERED: October 1, 1981 to Septmeber 30, 1982

TITLE OF PROJECT (80 characters or less)

A simple Microdiffusion Technique for Measuring Fluoride in Biological Samples

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR
Kennedy, John B.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Method Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This laboratory and two other laboratories assessed the fluoride content of aliquots from a pooled plasma sample which had been obtained from healthy individuals whose water supply was fluoridated (0.9ppm F). Agreement was noted between laboratories using the same method. However, the various methods detected different levels of fluoride in aliquots of plasma from the same sample.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DE 00323 02 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Natural transmission of Streptococcus mutans among rats consuming diets containing different concentrations of sucrose: induction of natural antibodies in serum and saliva.

NAMES, LABORATORY AND ISSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Stiles, Horace M.	Chief, E. Section	NCP CPR NIDR
Hso, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Groups of 12 weanling Osborne-Mendel rats were fed diets containing 0, 0.1, 1, 5 or 56% sucrose. Each rat was caged singly with a donor rat that was infected with Streptococcus mutans 6715-TS. The recipient and donor were swabbed daily and the bacteria were cultured on Mitis Salivarius agar.

Recipient rats fed 56 and 5% sucrose rapidly became infected with high levels of S. mutans. Recipients receiving low sucrose diets remained free of infection for much longer and when infected harbored lower numbers of organisms. The donor rats fed no sucrose failed to retain infection with S. mutans.

Antibody analyses await completion.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00324-02

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Methods of Analyzing Fluorosis Evaluations Made by Dean's Scoring System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kingman, A.	Statistician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dean's Scoring System has been used extensively in assessing fluorosis in the U.S. The traditional method of summarizing the level of fluorosis in a subject has been the Dean Index. Statistical methods of comparing the level of fluorosis in distinct communities using Dean's Index are being investigated. Other summary measures of fluorosis based on the Dean Scoring System are also being investigated together with the analytical methods of making comparisons among different communities.

Ridit analysis was used to compare the level of fluorosis in different communities using Dean's Index.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00325 02 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Streptococcus mutans transmission in rats consuming different concentrations of sucrose

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Stiles, Horace M.	Chief, P Section	NCP CPR NIDR
Cole, Michael F.	Acting Chief, E Section	NCP CPR NIDR
Li, Shou-Hua	Statistician (vis.)	NCP CPR NIDR
Waldrop, David	Computer Clerk	NCP CPR NIDR
Amsbaugh, Suzanne M.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods and Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thirteen litters of Osborne-Mendel 19-21 day old rats were distributed so that half of each litter would be in one group (Donors) and the other half in another group (Recipients). The recipient group was distributed one/cage for five groups of 12 rats each. Each of the 5 recipient groups, was placed on diets containing 0, 0.1, 1.0, 5.0 or 56 percent sucrose. All donors were placed on a diet containing 56 percent sucrose and inoculated intraorally with 0.2 ml suspension of Streptococcus mutans 6715-15; one ml of the suspension was placed in the drinking water of the donors. On day one donors were placed with recipients. The mouths of all rats were swabbed daily, the swabs placed in PBS, agitated and an laiquot was plated onto mitis salivarius ager. After 56 days blood and saliva weere collected from the rats at the time of sacrifice. Data are presently being assimilated for 1) dental caries development, 2) natural transmission of S. mutans from donor rats to recipients, on 5 different dietary concentrations of sucrose, 3) ability of sucrose to support initial S. mutans inoculation, and 4) ability of natural infection to induce a secretory response.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00327 02 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Human caries epidemiology among people consuming fluoridated/nonfluoridated hard and soft water

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Stiles, Horace M.	Chief, P Section	NCP CPR NIDR
Supan, Paul A.	Trainee	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous reports suggest a possible caries protective effect of hard water, even in the absence of fluoride. This study is designed to examine caries prevalence among 6-18 year old children consuming fluoridated and non-fluoridated water having four "degrees" of hardness. Other elements in the water supplies will be matched among the populations. Examinations will include a fluorosis index; plaque and saliva samples will also be collected for later analysis.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00328 02 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Role of transplacental IgG and colostral SIgA antibody in immune protection against Escherichia coli bacteraemia in neonatal rats.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

Bureau of Biologics - Dr. R. Schneeron, A. Sutton
University of Goteborg, Goteborg, Sweden - Dr. T. Soderstrom

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fourteen antibiotic suppressed Osborne Mendel female rats were divided into three groups. Four were immunized subcuteneously (SC) with 5 ug of K1-BSA three times at two week intervals. Four were immunized SC with 5 ug of Type I pilus and a final six with 5 ug of H18-BSA (Haemophilus influenza type 8 antigen). One week after the second immunization the rats were mated. After the dams delivered, the pups were divided such that groups of pups received antibody via the placenta alone, colostrum alone or by both routes. When the pups were six days old they were infected with 10^8 - 10^9 CFU of E. coli KRS203. Two days later bacteraemia was confirmed by plating blood samples onto ager plates containing antibody to K-1. Anti K1, Pilus and H18 antibodies in pup sera, and dam sera and colostra were analyzed by ELISA. No clear correletion was evident between serum antibody to K1 and pilus in the pups and/or colostral IgA and IgG anti K1 and pilus in the dams and protection against bacteraemia.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00342 01 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Clinical pharmacology study of the Intraoral Fluoride Releasing Device

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR

COOPERATING UNITS (if any)
John F. Kennedy Institute, Seth B. Canton
Johns Hopkins University, Charles J. Donnelly

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Md

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
This study will evaluate the performance and durability of two intraoral fluoride releasing devices with different release rates over a six month period and determine patient acceptance and tolerance of the devices over the same time span. Forty patient volunteers, aged 11-14 years, will be selected as participants and will be divided into 2 groups of 20. The study will consist of a 4 week pretreatment phase, a 26 week treatment phase, and a 4 week posttreatment phase. During the treatment phase, patients, according to their group assignment, will wear a device releasing either 0.05 or 0.10 mg of fluoride per day on their first maxillary molars. Periodically samples of saliva, dental plaque, urine, and serum will be collected to monitor fluoride levels and oral tissues will be monitored for any signs of irritation. Patients will be asked to use a fluoride-containing dentifrice but otherwise will be allowed to follow their normal oral hygiene procedures.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00343 01 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Pathogenesis in dental plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kemp, Christopher W.	Laboratory Technician	NCP CPR NIDR
Curtis, Michael A.	Laboratory Scientist (vis.)	NCP CPR NIDR
Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)
Hazelton Laboratories

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
This project is being conducted to demonstrate the presence of methanogenic bacteria in the dental plaque of monkeys (Macaca fascicularis and Macaca mulatta). The work completed to date using enrichment culture techniques has demonstrated the presence and metabolic activities of these methanogenic bacteria. The work is being directed towards the isolation of a pure culture of the methanogen(s) and a better characterization of the substrates utilized for methane production.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00344 01 CPR

PERIOD COVERED
October 1, 1981 to September 1, 1982

TITLE OF PROJECT (80 characters or less)
Mucosal immunity to Pseudomonas and Staphlococcus in patients with Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Hsu, Su-Cheng	Laboratoty Technician	NCP CPR NIDR

COOPERATING UNITS (if any)
NIADDK - Dr. V. McCarthy

LAB/BRANCH
Caries Prevention & Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Persons with Cystic Fibrosis (CF) are uniquely susceptible to respiratory infection with Pseudomonas aeruginosa and Staphlococcus aureus. In order to examine the immune response to these pathogens samples of saliva and serum were collected from CF patients and controls and assayed for antibodies reactive with these pathogens using a direct ELISA. Subjects with CF exhibited high antibody titers to Pseudomonas whereas control levels were low. The results thus far indicate that CF patients mount an immune response against these mucosal pathogens but are unable to eliminate them.

PHS-6040 (Rev. 8-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00345 01 CPR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Natural transmission of Streptococcus mutans in rats: Kinetics of induction of natural antibodies in serum and saliva

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Stiles, Horace M.	Chief, E Section	NCP CPR NIDR
Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
A group of 48 weanling Osborne-Mendel rats were fed diet containing 56% sucrose. Each rat was caged singly with a donor rat that was infectd with Streptococcus mutans 6715-15. The recipients and donors were swabbed twice a week and the bacteria cultured on Mitis Salivarius agar. Every two weeks a subgroup of 12 donors and 12 recipients were sacrified. The teeth were evaluated for caries and serum and saliva collected for antibody assay.

Samples await antibody assay.

PHS-6040 (Rev. 8-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00346-01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Rapid purification of rat SIgA and IgM from colostrum and serum using high performance liquid chromatography (HPLC)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Preventive Methods Development

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purification of immunoglobulins, particularly those of the rat, by classical methods is difficult and laborious. High performance liquid chromatography (HPLC) is a rapid technique capable of high resolution. Secretory IgA has been purified from defatted and decaseinated colostrum and IgM from the euglobulin fraction of serum in a single step. The proteins appear homogenous by immunoelectrophoretic analysis.

PHS-6040 (Rev. 3-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00347 01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Influence of metal ions on dental caries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Rolla, Gunnar R.	Laboratory Scientist (vis.)	NCP CPR NIDR
Afseth, John	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR
Amsbaugh, Suzanne M.	Laboratory Technician	NCP CPR NIDR
Levy, Donna	Student Volunteer	NCP CPR NIDR

COOPERATING UNITS (if any)

University of Oslo, Norway - Dr. J. E. Ellingsen

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Stannous ions and copper ions are known to inhibit dental plaque formation and reduce the acidogenicity of dental plaque in humans and in animals. In the present study copper ions reduced caries in rats when applied topically or added to the drinking water; this appeared to be related to the decreased numbers of S. mutans in the animals. Stannous ions, supplied as stannous fluoride, also reduced both caries and numbers of S. mutans. Stannous tartrate and stannous chloride did not reduce dental caries in rats; these results may be due to the low concentration of stannous ions available at the acidic pH values of the test solutions.

PHS-6040 (Rev. 2-81)

SMITH-SONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00348 (01) CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Purification of cell wall protein antigens of Streptococcus mutans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Little, Wayne A.	Laboratory Technician	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is in its initial stages, and purification procedures, as presented in the literature, are being evaluated and modified. Rabbits have been immunized with SDS treated cells of strain Ingbritt to produce antisera reported to react only with A and B protein antigens. Crude protein fractions have been prepared by growing cultures in ultrafiltrated Jordan's Strep Broth under controlled pH conditions. Culture fluids have been concentrated 100 fold in preparation for gel filtration of protein components. The use of affinity chromatography columns for removal of glucosyltransferase (GTF) has been investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00349 01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of sugar substitutes on bacterial growth, fermentation and polysaccharide synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Rolla, Gunnar R.	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Sonju, Torleif	Laboratory Scientist (vis.)	NCP CPR NIDR
Nagorski, Kathleen	Student Volunteer	NCP CPR NIDR
Wilbanks, Jennifer	Laboratory Technician	NCP CPR NIDR
Hoppes, Charles	Laboratory Technician	NCP CPR NIDR
Levy, Donna	Student Volunteer	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of the sucrose derivatives, lylose (palatinose) and palatinit, and of the artificial sweeteners saccharin and aspartame, on the growth, acid production, and glucan synthesis by oral streptococci were assessed. The four sugar substitutes were not fermented by any of the test bacteria. Saccharin inhibited growth and acid production by strains of S. mutans, S. sanguis, and S. salivarius grown in the presence of either sucrose or glucose. Glucan synthesis by S. mutans glucosyltransferase was inhibited by saccharin, palatinose and palatinit; inhibition was non-competitive with sucrose.

The uptake of ^{14}C-xylitol and the effect of xylitol on the uptake of ^{14}C-glucose by S. mutans, S. sanguis, S. salivarius and S. mitis were studied in resting cell cultures. Xylitol did not significantly effect the rapid uptake of ^{14}C-glucose by the oral streptococci. However, ^{14}C-xylitol was taken up at a slow rate by several strains of bacteria. The major metabolite of ^{14}C-xylitol extracted from these cells was tentatively identified as xylitol-5-phosphate.

PHS-6040 (Rev. 3-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 201 DE 00350 01 CPR

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Quantitation of peptostreptococci in dental plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Curtis, Michael A.	Laboratory Scientist (vis.)	NCP CPR NIDR
Little, Wayne A.	Laboratory Technician	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR
Kemp, Christopher A.	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

Litton Bionetics

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the extent of the peptostreptococcal population in dental plaque from humans and experimental animals at different sites in the mouth. Polyclonal antibodies have been raised against whole cells of both a stock ATCC peptostreptococcal strain and an oral isolate provisionally identified as a peptostreptococcus. Preliminary results show little cross reactivity with other oral microorganisms and also little strain cross reactivity. The peptostreptococcal population of plaque will be examined using fluorescently labelled antibody.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 201 DE 00351 01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Proline reduction in dental plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Curtis, Michael A.	Laboratory Scientist (vis.)	NCP CPR NIDR
Kemp, Christopher W.	Laboratory Technician	NCP CPR NIDR
Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

Hazelton Laboratories

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the mechanisms and effects of the reduction of proline to δ-NH_2 valeric acid in dental plaque from monkeys. Incubation of dental plaque homogenates with a variety of substrates suggests that proline is converted to δ-NH_2 valeric acid by means of Stickland reactions involving other amino acids and also certain end products of glucose metabolism notably pyruvic and lactic acids. The work is being directed towards elucidating the mechanism and products of these reactions and their contribution to the overall metabolism of dental plaque.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 201 DE 00352 01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The metabolism of the amino acid fermenting organisms of dental plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Curtis, Michael A.	Laboratory Scientist (vis.)	NCP CPR NIDR
Kemp, Christopher W.	Laboratory Technician	NCP CPR NIDR
Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to examine the metabolism of amino acids, particularly proline, by anaerobic organisms found in dental plaque. An organism isolated from saliva, which requires proline as an essential nutrient has been investigated in pure culture. Results, so far, suggest that the metabolism of this organism may involve the utilization of "Stickland reactions" involving both amino acids and lactic acid as substrates for the reduction of proline. Preliminary characterization suggests the organism is a member of the Peptostreptococci. The work is being directed toward the characterization of the kinetics and mechanisms of these reactions and their effects on dental plaque biochemistry.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 201 DE 00353-01

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

A more efficient use of Initial DMFS in Predicting Dental Caries Incidence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kingman, A.	Statistician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Prediction models for the development of dental caries have been investigated by many researchers. To date the variables shown most often to be correlated with the development of dental caries are the subject's DMFS prevalence and the number of permanent surfaces that exist in the dentition. In a previous study Kingman has shown that the incorporation of the various surface susceptibility levels for caries into the subject's caries prevalence assessment can improve models used for caries prediction.

The current investigation focuses on differences in surface susceptibility levels to caries attack and also the separate counts of D, F and M surfaces diagnosed for the subject. A weighted caries experience is derived using these factors and tested for its predictive power.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DE 00354-01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Statistical methods of analyzing microbiological changes in plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Li, shou-Hua	Statistician (Vis.)	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to investigate different statistical methods of analyzing microbiological changes in plaque. There are three different approaches to the study of this situation. The simplest approach is through the use of univariate analysis of variance. The second approach is based on the multivariate analysis of variance technique. A third approach is based on the polynomial growth curve model, i.e. a separate polynomial growth curve is fit for each group. All three different methods will be performed on the recent NCP experiment -- "Natural transmission of streptococcus mutans among rats consuming diets containing different concentrations of sucrose." This study is being undertaken to attempt to evaluate the advantages and disadvantages of different statistical methods of analyzing microbiological changes in plaque.

PHS-6040 (Rev. 8-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DE 00355 01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Sucrose mediated polysaccharide formation in human saliva-clinical implications.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Rolla, Gunnar R.	Laboratory Scientist (vis.)	NCP CPR NIDR
Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Afseth, John	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Schultz, Sandra	COSTEP (Dental)	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Free glucosyltransferase (GTF) and fructosyltransferase (FTF) were shown to be present in the salivas of several test individuals. Both enzymes were taken up from saliva by hydroxyapatite. Sucrose rinses increased the levels of GTF in saliva measureably. It appears that GTF can absorb directly to teeth independent of bacteria and thus could influence bacterial adsorption in the tooth pellicle.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DC 00356-01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Host proteins and bacterial products in the acquired enamel pellicle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Rolla, Gunnar R.	Laboratory Scientist (vis.)	NCP CPR NIDR
Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hydroxyapatite powder (HA) was coated with human whole saliva and injected subcutaneously into rabbits. The resulting antisera were reacted with commercial preparations of proteins by immunodiffusion and immunoelectrophoretic methods for tentative identification of antigens in the saliva coat on HA. Saliva proteins were eluted from the HA with phosphate buffer and reacted with specific antiserums using immunological techniques. Specific chemical and enzymic methods were also employed for identification of the coat proteins. IgA, IgG, albumin, lysozyme, α-amylase and bacterial glucosyltransferase were identified as proteins present in the saliva coat on HA. Many, if not all, of these proteins could be involved in the colonization of oral bacteria.

PHS-6040 (Rev. 8-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 DE 00357 01 CPR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less): Hydrophobic interactions in dental plaque formation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Sonju, Torleif	Laboratory Scientist (vis.)	NCP CPR NIDR
Rolla, Gunnar R.	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Lau, Agnes	COSTEP (Dental)	NCP CPR NIDR
Hoppes, Charles	Laboratory Technician	NCP CPR NIDR
Forquer, Kelly	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology Section

INSTITUTE AND LOCATION: NIH, NIDR, Bethesda, MD 20205

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cell surface hydrophobicity of strains of S. sanguis, S. salivarius, S. mitis and S. mutans was assessed by measuring: bacteria adsorption to polystyrene and glass surfaces; partitioning of ^{3}H-labeled bacteria in hydrophobic-hydrophilic two phase systems consisting of hydrocarbon-water, polyethylene glycol-dextran, and ficoll-dextran; and adsorption of ^{3}H-labeled bacteria to butyl- and octyl-agarose. The contribution of hydrophobic interactions to bacterial affinity for solid surfaces was demonstrated by increasing the salt concentration to 3M sodium chloride, which increased adsorption, and by adding potassium thiocyanate, a chaotropic agent, which decreased adsorption. S. sanguis cells showed the strongest and S. mutans cells the weakest tendencies to undergo hydrophobic bonding. Pretreating surfaces with human saliva tended to reduce strong adsorption caused by hydrophobic interactions. However some saliva coated S. mutans strains showed an increased tendency to undergo hydrophobic interactions. The potential of oral streptococci for hydrophobic interactions with solid surfaces in vitro thus appears significant. The contributions of such interactions in bacterial colonization of oral surfaces in vivo needs to be evaluated.

PHS-6040 (Rev. 8-81)

Part C

NATIONAL INSTITUTE OF DENTAL RESEARCH ANNUAL REPORT

Extramural Programs

October 1, 1981 - September 30, 1982

EXTRAMURAL PROGRAMS

NATIONAL INSTITUTE OF DENTAL RESEARCH

October 1, 1981 - September 30,1982

REPORT OF THE ACTING ASSOCIATE DIRECTOR

The Extramural Programs of the National Institute of Dental Research support a wide spectrum of research ranging from laboratory investigations on the basic causes of oral diseases to clinical trials of new methods of treatment and prevention. This broad array of scientific activity is divided into five categorical programs, each supporting a specific area of dental research, and a non-categorical program which consists of five university-based Dental Research Institutres and Centers conducting research in the entire field of oral biology. The health scientist administrators who develop and manage these extramural programs encourage a high level of interest in oral biology throughout the scientific community. In the planning and implementation of specific research programs, they carefully consider recommendations from advisory committees, special consultants and other NIH staff; cost factors are given due consideration. As a result of this collaboration, the NIDR Extramural Programs (NIDR/EP) have been able to maintain strong research programs with a reasonable level of funding. These programs have set the stage for the currently expanding clinical research programs in the fields of periodontal disease, oral soft tissue diseases, dental implantology, behavioral science and oral pain. The progress achieved in these areas is reflected in this report.

Personnel and Administration

During FY 1982 several important personnel changes took place. Towards the end of the year Dr. Richard L. Christiansen left his position as Associate Director, NIDR/EP, to assume the deanship of the University of Michigan School of Dentistry; and Dr. E. L. Rigg left his position as Chief of the Scientific Review Branch to assume the position of Acting Associate Director of NIDR/EP. Dr. H. George Hausch was asked to serve as Acting Chief of the Scientific Review Branch and to continue his responsibilities as Chief of the Office of Centers and Special Programs. In view of Dr. R. J. Schuellein's plans for retirement, Dr. Anthony A. Rizzo was asked to assume Dr. Schuellein's responsibilities as Special Assistant for Research Manpower. Personnel changes also included the departure of Dr. David Wolff, who left his position as a program officer in the Soft Tissue Stomatology and Nutrition Program to assume a position in the National Institute of General Medical Sciences, and the advent of Dr. John D. Suomi to serve as a program officer in the Craniofacial Anomalies Program.

In another move which involved changes in both administration and personnel, the Office of Collaborative Research was abolished and a new section called the Contracts Management Section was set up within the NIDR/EP. Ms. Edith Mullen was appointed Chief of this new section and Ms. Marian Blevins serves as Contract Specialist. In addition, Dr. Eugene L. Walter, formerly Chief of the Contract Review Section within the Office of Collaborative Research, was appointed to the Scientific Review Branch of the NIDR/EP as a Review Officer, but continues to maintain his responsibilities for the review of contract proposals. Altogether, 5 individuals formerly within the Office of Collaborative Research have joined the staff of NIDR/EP.

The Scientific Review Branch continued its usual activities and in addition, assumed the responsibility of review for the Small Grants program initiated this past year. In FY 1982,staff of this Branch conducted ten project site visits and convened three meetings of the NIDR Special Grants Review Committee, which reviewed ten Institutional National Research Service Award (NRSA) applications requesting $3.1 million, two Periodontal Clinical Research Center applications requesting $4.5 million, one Dental Research Institute application requesting $9.8 million and 162 Small Grant applications requesting $2.3 million. The Scientific Review Branch also conducted six No-Study-Section reviews. In addition, staff prepared 181 initial review summary statements for secondary review by the National Advisory Dental Research Council.

The Office of Centers and Special Programs provided fiscal and administrative support for the five non-categorical Dental Research Institutes and Centers and provided staff to work with representatives of the Division of Research Resources (DRR) on the Minority Biomedical Support (MBS) Program. The Chief of this office also administered Short Term Training grants, served as the NIDR liaison to the National Institute on Aging (NIA) in the administration of the Geriatric Dentistry Academic Awards, and served as the NIDR

representative to DRG to resolve problems of assignment and review.

Staff Activities

The Special Assistant for Research Manpower served as Chairman of the Staff Executive Committee, which provides secondary review of Fellowship applications, and of the Fellowship Advisory Committee, which advises the Director, NIDR, on NRSA Fellowship policies and practices. In addition, he prepared various documents on the implementation of the manpower provision of the Omnibus Reconciliation Act of 1981, on current trainee costs, and on projected estimates of future training needs. He also served as Executive Secretary of the Dental Research Institute and Special Programs Advisory Committee when it reviewed institutional fellowship grant applications; conducted site visits for program projects, center applications and research grants; and conducted No-Study-Section reviews of conference grant applications.

The Special Assistant for Program Coordination served as editor of the NIDR/EP annual reports and written material prepared for various purposes such as the Congressional Budget Justification, the NIDR Research Plan, and responses to Congressional inquiries. As the official representative of NIDR, he served as a member of the Diabetes Mellitus Interagency Coordinating Committee, the Office of Medical Applications Research Committee (now called the CCATT Committee) and attended meetings of the National Diabetes Advisory Board. Towards the end of the year, he assumed the administrative responsibilities of the Special Assistant for Research Manpower (as described above).

Extramural program staff made 25 visits to institutions and participated in 24 different scientific meetings to keep abreast of scientific developments, monitor research progress, and maintain close liaison with the scientific community. In addition to their informal contributions to these activities, staff made formal presentations at some meetings and participated in the preparation of the proceedings for publications. They also attended 10 courses for professional development. Information on program priorities for federal funding was again disseminated by staff at the annual meetings of the American Association of Dental Schools, and of the IADR/AADR in New Orleans. At these meetings staff members made several formal presentations and participated in the activities of the various specialty groups within the IADR. At the IADR/AADR meeting, staff again maintained a consultation room to inform scientists of research opportunities, resolve problems, and encourage young investigators to develop research plans.

Meetings Sponsored.

In FY 1982 NIDR awarded grants to partially support three scientific meetings. One of these provided support for the April 1982 annual joint meeting of the International Biomaterials Symposium and Society for Biomaterials; a second provided funds for the 1982 Gordon Conference on Bones and Teeth; and the third grant provided travel support for NIDR grantees and contractors to attend the 1982 IADR/AADR meeting in New Orleans.

Centers

The NIDR supports eight centers. Five of these are non-categorical, university-based centers initiated during the 1960s; and three are specialized centers initiated more recently to accelerate clinical research on periodontal diseases.

The non-categorical Dental Research Institutes and Centers (DRIC) at the Universities of Alabama, Michigan, North Carolina, Pennsylvania, and Washington supported 81 research projects during FY 1982. Although the level of their activities continued to decline because of reduced funding and increased costs, these centers have maintained an outstanding record of scientific achievement. During the past year, their senior investigators published 327 scientific papers (excluding abstracts) and had an additional 127 papers accepted for publication. Research training was provided for 39 research associates through direct participation in the research activities, and Center investigators also served as preceptors for 93 fellows, many of whom conducted their research in the DRICs.

During this past year, site visits were made to three of the five centers. A site visit to the University of Alabama Institute of Dental Research was conducted as a part of the review of this center's five-year renewal application. The Alabama center was found to be highly productive and was approved for an additional five years. In addition, an interim project site visit to the University of Michigan center was conducted and a staff visit was made to the University of Washington center. Notwithstanding the continuing effects of budgetary constraints resulting from increased costs in the face of reduced funding, the research programs of these were judged to be progressing satisfactorily.

During FY 1982 activities leading to an eventual evaluation of the centers continued. A comprehensive

compilation and cataloguing of administrative data from the official files of the DRIC program was completed under contract; and plans were initiated for a citation analysis of DRIC publications, to be conducted under contract.

Research highlights have not been described in this administrative overview of the DRIC program. Instead, they are included in the categorical program reviews which follow.

The three Specialized Clinical Research Centers for Periodontal Diseases at Forsyth in Boston, SUNY at Buffalo and Virginia Commonwealth University in Richmond have continued to make significant progress in clinical, microbiological and immunological studies related to periodontal diseases. According to recent investigations at the Centers, the concept that periodontal diseases involve a slow, continuous, progressive disease process is no longer tenable. The Center investigators have used three analytical procedures to distinguish active diseased sites from inactive ones in human subjects. These highly significant studies indicate that destructive periodontal disease activity occurs in discrete "bursts" rather than as a continuous process. Another technical breakthrough is the demonstration of local antibodies to periodontopathic organisms in the crevicular fluid of periodontal pockets. These findings are expected to provide better preventive, diagnostic and treatment procedures.

The Small Grants Program, briefly described in last year's annual report, was successfully initiated during FY 1982. This NIDR award provides up to $15,000 over a 2-year period for small projects to determine the feasibility of a larger study, to develop new research techniques, to study a special clinical problem, or to analyze existing data. During FY 1982, the first round of applications was completed and 15 awards were made. The new award is expected to fulfill an important need in the development of significant projects to be funded by regular grants in the future.

Research Funding

During FY 1982 the NIDR Extramural Programs awarded research funds of $37 million, which included $29.2 million for research grants and career awards, $0.8 million for contract research by two of the five categorical programs, and $7.0 million for the 5 university-based Dental Research Institutes and Centers.

GRANTS

Table 1 presents data on the FY 1982 distribution of research funds by program and by type of grant. It does not include grants by the National Caries Program. Altogether, the Extramural Programs made 330 grant awards: 297 for research projects, 3 for scientific conferences, 5 for the university-based dental research institutes and centers, 3 for periodontal clinical research centers, 21 for research career development awards, one for a research career award. Of the 297 project awards, 232 were made for regular research grants (R01), 8 for program projects (P01), 42 for new investigator awards (R23), and 15 for small research grants (R03).

In FY 1982 25% of the research grant funds was awarded for new grants and competing renewals, and 75% was awarded for noncompeting continuations and supplemental grants. The new awards included 28 regular grants, 13 new investigator awards, and 15 small grants. The competing renewals included 36 regular grants, one program project, and 2 periodontal clinical research centers.

Table 2 shows the levels of research funding by each Program compared to the previous year and the percentage increase or decrease. Although there was an overall 3% increase in funding for NIDR/EP, individual program funding varied considerably. Three programs showed significant increases, and two showed significant decreases.

R154

N.I.D.R. EXTRAMURAL RESEARCH GRANTS FUNDED FY 82
DATA AS OF SEPTEMBER 30, 1982 - BY TYPE & CODE

TYPE/ CODE	PERIODONTAL NO.	PERIODONTAL AMOUNT	CRANIOFACIAL NO.	CRANIOFACIAL AMOUNT	RESTORATIVE NO.	RESTORATIVE AMOUNT	STOMATOLOGY NO.	STOMATOLOGY AMOUNT	PAIN CONTROL NO.	PAIN CONTROL AMOUNT	INSTITUTES NO.	INSTITUTES AMOUNT	TOTALS NO.	TOTALS AMOUNT
1 K04	2	$76,036	1	$39,118	0	$0	0	$0	0	$0	0	$0	3	$115,154
R01	5	$641,652	5	$402,815	5	$267,192	7	$584,515	6	$677,335	0	$0	28	$2,573,509
R03	5	$69,267	5	$73,792	1	$21,149	3	$44,098	1	$14,600	0	$0	15	$222,906
R13	0	$0	0	$0	2	$87,414	1	$5,000	0	$0	0	$0	3	$92,414
R23	5	$262,500	1	$52,030	0	$0	4	$182,236	3	$144,868	0	$0	13	$641,634
TYPE TOTALS	17	$1,049,455	12	$567,755	8	$375,755	15	$815,849	10	$836,803	0	$0	62	$3,645,617
2 P01	0	$0	1	$384,811	0	$0	0	$0	0	$0	0	$0	1	$384,811
P50	2	$1,083,839	0	$0	0	$0	0	$0	0	$0	0	$0	2	$1,083,839
R01	7	$970,517	6	$597,367	5	$442,375	13	$1,624,673	5	$480,305	0	$0	36	$4,115,237
TYPE TOTALS	9	$2,054,356	7	$982,178	5	$442,375	13	$1,624,673	5	$480,305	0	$0	39	$5,583,887
3 R01	0	$40,731	0	$0	0	$0	0	$0	0	$0	0	$0	***	$40,731
TYPE TOTALS	0	$40,731	0	$0	0	$0	0	$0	0	$0	0	$0	***	$40,731
5 K04	5	$184,052	3	$114,319	2	$77,890	5	$186,030	3	$117,062	0	$0	18	$679,353
K06	0	$0	0	$0	0	$0	1	$32,670	0	$0	0	$0	1	$32,670
P01	1	$610,564	5	$2,035,999	0	$0	1	$206,677	0	$0	0	$0	7	$2,853,240
P50	1	$529,160	0	$0	0	$0	0	$0	0	$0	5	$7,086,000	6	$7,615,160
R01	36	$3,161,755	44	$3,847,378	26	$2,435,856	42	$3,395,523	20	$1,517,642	0	$0	168	$14,358,154
R23	6	$290,210	5	$216,603	5	$211,956	8	$437,720	5	$205,192	0	$0	29	$1,361,681
TYPE TOTALS	49	$4,775,741	57	$6,214,299	33	$2,725,702	57	$4,258,620	28	$1,839,896	5	$7,086,000	229	$26,900,258
GRAND TOTALS	75	$7,920,283	76	$7,764,232	46	$3,543,832	85	$6,699,142	43	$3,157,004	5	$7,086,000	330	$36,170,493

THIS REPORT DOES NOT INCLUDE THE NATIONAL CARIES PROGRAM.

TYPES:
1 NEW AWARDS
2 COMPETING CONTINUATION AWARDS
*** 3 SUPPLEMENTAL AWARDS
5 NON-COMPETING CONTINUATION AWARDS

CODES:
K04 CAREER DEVELOPMENT AWARDS
K06 CAREER AWARDS
P01 PROGRAM PROJECTS
P50 INSTITUTE AWARDS
R01 REGULAR RESEARCH GRANTS
R03 SMALL RESEARCH GRANTS
R13 CONFERENCE GRANTS
R23 SPECIAL DENTAL AWARDS

Table 2. Comparison of Funding by Program and Previous Year

Program	FY1981 ($000s)	FY1982 ($000s)	Increase or (Decrease)	% Change
Periodontal	$7054	$7920	$866	12.3
Craniofacial	8076	7764	($312)	(3.9)
Restorative	3575	3544	($ 31)	(0.9)
Soft Tissue	5927	6699	$772	13.0
Pain & Behavior	2921	3157	$236	8.1
Five Centers	7476	7086	($390)	(5.2)
Totals	$35,030	$36,170	$1141	3.0

CONTRACTS

Collaborative research funded by contract or by interagency agreement during FY 1982 consumed $851 thousand, a 21% decline from the FY 1981 level of $1.1 million. More than 90% of these funds were spent for interagency agreements to support laboratory and clinical research in restorative materials. The laboratory research dealt with bonding agents and with materials for dental fillings and prosthetic appliances. The clinical research involved the long-term evaluation of dental restorations and efforts to develop an improved dental radiographic system for diagnosis. The remaining funds supported a project in the craniofacial area.

TRAINING

The distribution of NIDR research training funds awarded in FY 1982 is summarized in Table 3. A total of 91 awards were made at a cost of $3.5 million, a decline of $0.9 million or 20% from the $4.4 million awarded during FY 1981. Included in these awards were 43 individual fellowships, 25 institutional fellowship grants, 5 senior fellowships and 15 short-term training grants. The 25 institutional grants provided 26 predoctoral and 115 postdoctoral trainee positions, but only 20 predoctoral trainees and 95 postdoctoral trainees actually received training during FY 1982. Thus, approximately, 20% of the positions remained unfilled. During FY 1982, short-term training grants to dental schools were expanded from 15 grants supporting 95 fellows (FY 1981) to 16 active grants supporting 103 fellows; and the funding of the 6 active Geriatric Dentistry Grants to dental schools by the National Institute on Aging (NIA) was maintained. The NIDR provided consultation during the initiation and development of these awards. Recipients of these awards are expected to develop an improved curriculum on geriatric dentistry and to initiate research programs. After FY 1983 these awards will no longer be available.

The NIDR has also participated in efforts to provide a broad range of training opportunities for minority students through cooperative agreements with other NIH organizational components. During FY 1982, as in the previous year, 4 students received NIDR training funds under the Minorities Biomedical Support (MBS) Program through an agreement with the NIH Division of Research Resources (S06 awards). A second continuing agreement with the National Institute of General Medical Sciences provides for the support of postdoctoral dental fellows through the Minorities Access to Research Careers (MARC) Program. During FY 1982, NIDR provided support for two fellowships under this program (F34 awards).

During FY 1982 training support was provided for a total of 272 individuals, including 169 individuals receiving full-time support, and the 103 individual short-term fellows. The number being supported full-time declined by 13% from the FY 1981 total of 194 individuals.

Table 3: Distribution of NIDR Research Training Funds in FY 1982 ($ in Thousands)

Type of Award	Caries		Perio.		Cranio.		Restor.		Stomat.		Pain		Total	
	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.
Individual Fellowships	6	$105	14	$ 250	11	$186	2	$ 40	6	$105	4	$ 76	43	$ 763
Institutional Grants	2	142	6	890	6	534	4	303	4	242	3	234	25	2,345
Senior Fellowships	1	33	-	--	2	59	-	--	1	24	1	33	5	149
Short-Term* Tr. Grants	-	--	1	7	4	58	5	52	-	--	5	56	15	173
Minority Awards (F34, S06)	2	83	1	14	-	--	-	--	-	--	-	--	3	97
Totals	11	$363	22	$1,161	23	$837	11	$395	11	$371	13	$399	91	$3,527

*Non-categorical grants assigned to Programs for budgetary reasons only.
One additional Short-Term grant was active, but received no funds in FY 1982.

PERIODONTAL DISEASES PROGRAM

Introduction

Periodontal diseases pose a major threat to human health throughout the world. Millions of Americans have already lost their teeth from these diseases and millions more will become edentulous unless effective preventive measures can be developed. The steadily increasing longevity of the population makes the control and prevention of periodontal diseases even more urgent and challenging for public health workers and researchers. The goal of the Periodontal Diseases Program is to eradicate these diseases. To achieve this goal, the Program supports research on the cause, nature, diagnosis, treatment, and prevention of these diseases. Because the etiology of periodontal diseases is multifactorial, the research encompasses a wide variety of subject matter, which includes microbiologic studies on the identification and nature of the suspected pathogens, immunologic studies in the complex host response system activated by the disease, as well as studies to develop new therapeutic approaches. The microbiologic studies emphasize anaerobic bacteria and the host response studies emphasize the cellular and biochemical mechanisms involved in inflammation and tissue destruction. The research on new therapeutic approaches involves coordinated clinical and laboratory studies to develop practical preventive measures suitable for the general public.

Administration

During FY 1982 the Program awarded a total of $7,920,283 for 48 regular research grants, 3 specialized clinical research centers, one program project, 11 young investigator research awards and 5 small grants. Another 11 regular research grants and 2 young investigator research grants were active but did not receive FY 1982 funds. A total of $897,265 was awarded for 7 institutional training grants supporting 44 fellows and an additional $264,086 was awarded for 15 individual fellowships. A total of $260,088 was expended for 6 career development awards.

Awards for the 3 specialized centers established to develop coordinated programs of basic and clinical research on periodontal diseases totaled $1,612,999 in FY 1982. At these centers the identification and characterization of the oral microflora and the elucidation of the specific host response to certain pathogenic organisms are the main thrusts of research. In addition, efforts are also being made to develop improved therapeutic and preventive measures. In spite of a reduction in FY 1982 funding, the three clinical research centers continued to make commendable progress.

Table 1 shows the distribution of research and training funds during FY 1982 according to subject category.

Table 1 DISTRIBUTION OF FUNDS DURING FY 1982

A. RESEARCH GRANTS

	Active	Funded	Funds ($000s)	Percent
Microbiology	25	22	$2,298	29.0
Inflammation and Immune Response	26	22	1,519	19.2
Bone Metabolism	8	7	655	8.3
Periodontal Tissue Structure & Metabolism	12	9	974	12.3
Prevention, Diagnosis, and Treatment	7	6	602	7.6
Clinical Research Centers	3	3	1,613	20.4
Career Development Award	7	6	260	3.3
Totals	88	75	$7,920	100.1
B. TRAINING				
Institutional Grants	8	7	897	77.3
Individual Fellowships	20	15	264	22.7
Totals	28	22	$1,161	100.0
Grand Totals			$9,081	

Staff Activities

Staff visited 8 institutions to program, monitor, and evaluate research; and attended 6 meetings to keep abreast of scientific developments and to maintain close liaison with the scientific community.

A. Site Visits — Initial Review, Monitoring, and Programming

University of Pennsylvania, Philadelphia	October 1981
State University of New York, Buffalo & Gila Indian Reservation, Phoenix	December, 1981
University of Nebraska, Lincoln	January, 1982
Virginia Commonwealth University, Richmond	February 1982
Fairleigh Dickinson University	April 1982
University of Michigan, Ann Arbor	June 1982
Forsyth Dental Center, Boston	August 1982

B. Meetings

American Academy of Periodontology, Annual Meeting, Toronto	October 1981
NIDR Long Range Plan Coordinating Committee, Bethesda	November 1981
International Association for Dental Research, New Orleans	March 1982
District of Columbia Dental Society Annual Meeting, Washington, D. C.	April 1982
5th International Conference on Periodontal Diseases, Seattle	July 1982

C. NIDR Programs Advisory Committee Annual Meeting, Bethesda — May 1982

D. Subcommittee on Periodontal Diseases, Bethesda — December 1981, May 1982

Research Highlights

The research highlights outlined in this report were derived from studies in clinical periodontology, in oral microbiology and immunology and in basic connective tissue and bone metabolism. The clinical research section describes investigations to identify active lesions and studies related to the evaluation of different antibiotics for treatment. The microbiology section includes systematic study of microflora in experimental gingivitis and the identification of several new species of oral bacteria. The immunology section includes studies related to the development of techniques to measure crevicular fluid antibody to suspected pathogens and studies of neutrophil function in periodontal disease. Finally, the bone and connective tissue section outlines how mononuclear phagocytes are attracted towards the sites of bone resorption by the chemoattractants and the mechanism of phenytoin in producing the gingival overgrowth.

CLINICAL STUDIES

Investigators at Forsyth are using several methods to test the validity of their assumption that periodontal disease is characterized by intermittent exacerbation and remission rather than by slow and constant progression. To detect periods of destructive disease activity in individual sites they evaluated three analytical methods: regression analysis, the running median method and the tolerance method. The data in this study were based on pairs of repeated measurements of attachment level made at 6 sites on every tooth in 22 individuals with radiographic evidence of periodontal destruction. A total of 3,414 sites were monitored at 2 month intervals for up to 16 months. The results show that regression analysis is best suited to detect gradual steady changes in attachment levels, but not abrupt changes. The technique of running median is able to detect abrupt changes and cycles. It is well adapted to the task of monitoring the history of a site, especially when only single probe measurements are available. The tolerance method has the greatest potential for early detection of attachment level changes of any of the methods tested. This method not only considers the measurement variation in the population, but also the variation among specific sites within the individual subject. Based on the longitudinal studies of attachment levels and alveolar bone loss in patients and animals, the Forsyth group suggest that periodontal disease progresses by recurrent acute episodes. They believe that bursts of activity occur for short periods of time in a random fashion at periodontal sites throughout the mouth. Comparison of monitored rates of attachment loss for a year with mean rates of loss prior to monitoring suggest that there may be relatively short periods in an individual's life in which many sites undergo periodontal destruction followed by extended periods of remission. The possibility that destructive periodontal disease activity occurs in discrete bursts rather than as a continuous process is likely to influence both experimental design and patient therapy.

The rationale for the use of antibiotics in the management of periodontal disease is based upon the hypothesis that the various forms of periodontal disease are associated with specific groups of microorganisms. The investigators at SUNY, Buffalo, have evaluated the susceptibility of the microflora associated with periodontal lesions to a large number of antibiotics by using *in vitro* techniques. The plaque samples taken from lesions of patients with periodontal diseases were cultured on non-selective media with antibiotics in different concentrations and without antibiotics. A comparison of the counts of the antibiotic-containing versus non-antibiotic containing plates allowed one to estimate the antibiotic sensitivity of the flora in general. Further characterization and classification of the antibiotic resistant strains also provided information on the common resistant strains residing in the subgingival area. Even though most of the antibiotics tested were effective in inhibiting a majority (95%) of the subgingival flora at 0.1 microgram per ml there was considerable variation in the effectiveness of each antibiotic against specific bacteria. In general penicillin was the most effective, followed by the tetracyclines, minocycline and doxycycline. The next group, consisting of erythromycin, carbenicillin, clindamycin and spiramycin, were not as effective as the penicillin and tetracycline group. Chloramphenicol and metronidazole were the least effective. *Streptococcus sanguis, S. mitis, Veillonella* sp. and a significant number of strains of *Actinomyces* were resistant to the tetracyclines. The penicillin-resistant organisms consisted of anaerobic vibrios, *Veillonella parvula*, and *Actinobacillus actinomycetemcomitans.* Interestingly, few black-pigmented *Bacteroides* were resistant to the tetracyclines and to penicillin. In general penicillin and the tetracyclines were the most effective for inhibiting subgingival plaque microflora.

The administration of minocycline alone for seven days to adults with moderate to severe periodontitis, resulted in improved gingival health with marked reductions in total bacterial counts and in the proportions of spirochetes. Minocycline administration with periodontal scaling and root planing also resulted in major, long-lasting shifts in the subgingival microflora. Scaling alone was least effective in changing the microflora. Hence, the study indicated that minocycline may be a useful adjunct in the treatment of periodontal disease since it resulted in long-term suppression of the subgingival microflora.

The investigators at SUNY, Buffalo, have found that thorough supragingival and subgingival scaling and root planing, averaging six hours of debridement per patient, and topical betadine treatment resulted in a reduction of the total subgingival bacterial counts and of the proportion of spirochetes in patients with localized juvenile periodontitis (LJP). Nevertheless, this treatment failed to reduce the counts of *A. actinomycetemcomitans* . Betadine application alone had little effect on the *A. actinomycetemcomitans* population. In contrast, systemic tetracycline suppressed *Actinobacillus* and *Capnocytophaga* to negligible levels in all pockets. These results indicate that some form of antimicrobial therapy, preferably by a systemic route may be necessary to eradicate or substantially suppress the periodontopathic microflora of LJP lesions.

Another group of investigators at SUNY,Buffalo, have developed a series of salicylamide antiplaque agents. Fifty-five such agents including dibromo, alkyl and acylsalicyloyl derivatives of various anilines, heterocyclic amines, benzylamines and alkylamines were synthesized and evaluated for their *in vitro* antibacterial activity against *Actinomyces viscosus.* Several nonhalogenated salicylanilides were found to exhibit higher levels of *in vitro* antibacterial activity against a number of *Actinomycetes* than did tribromsalan or fluorophene, two antibacterial agents which have been previously used in mouth rinses. Since the use of halogenated salicylanilides has been restricted by the FDA, interest was directed to the newly synthesized nonhalogenated derivatives and further studies with these derivatives are in progress to evaluate their antiplaque activities.

In another study of antiplaque agents, eight recently developed branched alkylbisbiguanides were also found to be substantive to saliva coated enamel and to be effective in *in vitro* plaque inhibition. Like their parent compounds alexidine and chlorhexidine, these bisbiguanide derivatives showed *in vitro* antibacterial activity against *Bacteroides* species, including *B. gingivalis, Fusobacterium nucleatum, Campylobacter, A. actinomycetemcomitans, Haemophilus aphrophilus*, anaerobic vibrios and *streptococci.* In general, the structure-activity studies indicated that drugs with increased lipophilicity demonstrated decreased activity against all test strains. However, within the isolipophilic series of drugs, increased activity paralleled increased length of the central methylene bridge. Furthermore, with comparable lipophilicity and bridge length, drugs with branched terminal groups were more active than those with unbranched terminal groups. Several of the newly synthesized branched alkylbisbiguanides appear to be potentially valuable agents in the control of the periodontal microflora. As with the nonhalogenated salicylanilides, the branched alkyl derivatives of bisbiguanides are under further investigation for use in the management of periodontal infection.

MICROBIOLOGY

Periodontal tissues harbor hundreds of species of both pathogenic and nonpathogenic bacteria. Yet the oral microbial flora in health and disease remain poorly defined. The investigators at Virginia Commonwealth University in collaboration with investigators at Virginia Polytechnic Institute and State University did a comprehensive study of the microbial flora associated with experimental gingivitis, a well-established model for studying the development of gingival inflammation.

The study was conducted on 4 adult males and samples were taken on the 4th, 11th and 26th day of experimental gingivitis. One hundred sixty-six bacterial species and subspecies were detected among 3,034 randomly selected isolates from 96 samples. The major finding of this study are the following: *A. naeslundii, A. odontolyticus, F. nucleatum, Lactobacillus* species *D-2, S. anginosus, V. parvula* and *Treponema* species *A* are the most likely etiologic agents of gingivitis. The greatest source of microbiological variation of the total flora observed was person-to-person differences in the floras. The next greatest source of variation was the inflammatory status of the sample sites. Person-to-person differences were smallest on experimental day 4 when sample collection was begun. As gingivitis developed and progressed, the flora became more diverse and complex. The sequential colonization by certain species of bacteria was reproducible and therefore is probably predictable. Variation was relatively small between replicate trials (two sites on the same tooth sampled the same day, or the same sites sampled more than once for the same time period).

Identification of bacterial isolates from periodontal specimens is difficult, time consuming and expensive. But accurate identification is necessary for determination of the physiologic properties of species that may play key roles in the initiation and progression of disease. The investigators at Virginia Polytechnic Institute and State University have recently described several new species of bacteria belonging to the genus *Bacteroides. B. Loescheii* isolated from periodontal pockets are obligately anaerobic, gram-negative, usually pigmenting, nonmotile, nonsporing rods that do not grow well in 10% bile and that ferment carbohydrates. They had previously been identified as *B. melaninogenicus* or *B. oralis* but have no DNA homology with the type strains of these two species. Two other new species *B. oris* and *B. briccae* are also obligately anaerobic, gram-negative, nonpigmenting

non-motile, non-spore forming rods that do not grow well in 10% bile and ferment carbohydrates. They had previously been identified as *B. ruminicola* subsp. *ruminicola* or *B. ruminicola* subsp. *brevis* but have no DNA homology with the type strains of these two species.

Even though spirochetes are ubiquitous in the periodontal pockets, what role these organisms play has not been fully elucidated. This stems from the fact that it is difficult to culture these organisms *in vitro* so as to validate pathogenicity by animal experiments or to demonstrate virulence factors *in vitro*. The investigators at Virginia Polytechnic Institute and State University have isolated treponemes from patients with localized juvenile periodontitis, patients with moderate and severe periodontal diseases, and from adults who had experimental gingivitis. Spirochetes are either absent or present in extremely low numbers in individuals with healthy gingiva and no clinical signs of periodontal disease. The spirochete isolates have been grouped into about 14 species of which only 2 are currently recognized species: *Treponema denticola* and *T. Vincentii*. The Virginia group have studied some of the nutritional requirements and physiology of these species. Both species require thiamine pyrosphosphate (TPP) for growth. Eight to ten species of oral bacteria were found to secrete TPP into the culture medium and could serve as a source of TPP for these treponemes in the oral cavity. *T. denticola* and *T. Vincentii* were also found to require the alpha globulin fraction of serum, but albumin, beta globulin and gamma globulin did not support growth. Delipified alpha globulin did not support growth but growth could be restored by the addition of long chain fatty acids. Oleic acid was the only fatty acid that was required by these treponemes and lysophosphatdylcholine was the only phospholipid in serum that was degraded by these treponemes. These studies show that treponemes have complex growth requirements.

Oral spirochetes are divided into three morphological groups based on cell size. The investigators at the University of Minnesota have identified another morphological characteristic that can be used to classify the oral spirochetes based on the helical configuration of the cell. Spirochetes that coil in a clockwise direction are right-handed and those that coil counter clockwise are left-handed. They have determined that *T. denticola*, an oral treponeme, believed to be avirulent is right-handed. In addition they have detected a number of other oral spirochetes with definite left-handed helices. It is of interest to note that the pathogenic spirochetes like *Treponema pallidum* are known to possess left-handed helices.

A. actinomycetemcomitans is a gram-negative bacterium which has been implicated as a causative organism in localized juvenile periodontitis. The investigators at SUNY, Buffalo, have studied 297 periodontal isolates of *A. actinomycetemcomitans* from 70 individuals and found that this species contains at least 3 distinct serologic types. Even though all three serotypes were found among the patients, a given individual was infected with only one serotype. The serotype-specific antigens were heat stable and appeared to be polysaccharides. Often these individuals showed high serum antibody to the serotype-specific antigens suggesting that these antigens may play an important role in the pathogenesis of periodontal disease.

The investigators at Forsyth have developed a modified enzyme-linked immunosorbant assay (ELISA) system for the identification of a large number of gram-negative subgingival flora. Characterization of pure cultures before identification is a time-consuming and tedious procedure which may take from 2 to 4 weeks and require 16 to well over 100 tests for each isolate. The new ELISA procedure may be carried out on large numbers of strains within 2 hours once the antisera are available and validated. Using the ELISA procedure the Forsyth group have shown that *B. gingivalis* and *B. melaninogenicus ss. intermedius* strains can be identified rapidly and easily. The ELISA technique was also used to distinguish the three sero types of *A. actinomycetemcomitans* and additional species of *Wolinella*. The ELISA method will accelerate identification of organisms from subgingival sites and permit larger numbers of microbial samples to be analyzed.

IMMUNOLOGY

Until recently most of the studies of the host antibody response to suspected periodontal pathogens have relied only on measurements of the levels of antibodies in serum. Although it was recognized that studies on the presence and concentration of antibody to periodontopathic microorganisms in the local crevicular fluid would provide more pertinent information about the disease, such studies have only recently become feasible. At Forsyth Dental Center both serum and crevicular fluid levels of antibody to a battery of oral microorganisms were determined using an enzyme-linked immunosorbant assay (ELISA). The microorganisms included: *A. actinomycetemcomitans, A. naeslundii, B. gingivalis, B. melaninogenicus ss. intermedius, S. sputigena, C. concisus, E. corrodens, F. nucleatum, S. mutans, S. sanguis* and *W. recta*. A group of periodontal patients who had a distinct evaluations of serum antibody to certain microorganisms also had high titers of antibodies to the

same species of microorganisms in their crevicular fluid. The ratio of antibody activity in crevicular fluid to that in serum was used to determine whether local antibody synthesis was occurring. Ratios significantly higher than one indicated local antibody synthesis and were found in approximately 8% of the 1,007 sites sampled. Responses to multiple microorganisms in individual crevicular fluid samples indicated that some sites demonstrated high levels of crevicular fluid antibody to more than one microorganism. The role of microorganisms in eliciting these local antibody responses was analyzed comparing the relative concentration of crevicular fluid antibody with the presence of that species of microorganisms in individual sites. Ninety-five sites in 15 patients were examined for crevicular fluid antibody level and the presence of the homologous microorganisms. In 81% of the sites the bacterial species detected was consistent with the level of local antibody detected. Preliminary studies show that after periodontal treatment the crevicular fluid from localized juvenile periodontitis patients show decreased levels of antibody to *A. actinomycetemcomitans* . Moreover, microbiological samples taken from the treated periodontal pockets show a corresponding absence of *A. actinomycetemcomitans* . These findings suggest that a combination of potentially pathogenic microorganisms and an accompanying local immune response may indicate that the tooth has a high risk of disease.

The investigators at the University of Pennsylvania have shown that extracts of *A. actinomycetemcomitans* produce a factor which suppresses both B & T lymphocyte activity. The purified factor has a molecular weight of 50,000, is heat-labile, and trypsin sensitive. It was conjectured that the suppression of both B & T cell functions may be mediated through a common mechanism such as activation of T-suppressor cells. Although the immunological mechanisms involved in periodontal disease are not clearly defined, it is reasonable to predict that suppressed host defense mechanisms may contribute to the pathogenesis of this disease.

In a study of bacterial-neutrophil interactions investigators at SUNY, Buffalo, were able to show that neutrophil chemotaxis could be inhibited by soluble bacterial products. Some of the major bacterial species from the oral cavity were assessed for their ability to produce chemotactic factors, or to inhibit chemotaxis of neutrophils. It was found that *Capnocytophaga, Bacteroides* sp. *A. actinomycetemcomitans* (grown under conditions where they do not produce a leukotoxin) and *F. nucleatum* produce factors which specifically inhibit neutrophil chemotaxis. There were at least two mechanisms detected for this inhibition. Factors isolated from *Capnocytophaga* inhibited chemotaxis, but did not inhibit random migration or binding of the chemotactic factor to the neutrophil surface. On the other hand, extracts of *Bacteroides, A. actinomycetemcomitans* and *F. nucleatum* inhibited binding of the chemotactic factor to the neutrophil, as well as chemotaxis. It is proposed that these factors which inhibit neutrophil chemotaxis may be operative at local sites of inflammation and may be important determinants of virulence. This argument is strengthened by the finding that organisms which are not implicated in the pathogenesis of periodontal disease, such as *S. sanguis* and *S. mutans* do not inhibit neutrophil chemotaxis.

The investigators at the National Jewish Hospital in Denver have shown that certain substances obtained from bacteria can enhance the ability of macrophages to kill microorganisms. Bacterial lipopolysaccharide and muramyldipeptide caused the macrophages to release increased levels of toxic oxygen radicals when the macrophages phagocytized the microorganisms. These workers had previously shown that production of oxygen radicals was essential for efficient killing of pathogenic microorganisms by macrophages and other phagocytic cells. When injected into mice, muramyldipeptide protected the animals against an otherwise lethal injection of the fungus *Candida albicans*. Current studies regarding the biochemical mechanisms that control oxygen radical production by macrophages are aimed at developing drugs that can either increase oxygen radical production to enhance resistance to infection, or decrease oxygen radical production to limit damage to the tissues by the highly toxic oxygen radicals. Such drugs would be valuable in controlling infection and tissue damage associated with periodontal diseases.

BONE AND COLLAGEN METABOLISM

The NIDR continues to support numerous studies on the structure and metabolism of the connective tissue and bone because these tissues are involved in periodontal diseases. The highlights for this year include studies on the role of monocytic phagocytes in bone resorption and investigations related to the breakdown of connective tissue by bacterial collagenase.

Several laboratories have presented evidence to show that mononuclear cells provide the precursor cells which become osteoclasts. Both osteoclasts and monocytes share a number of anatomical and functional characteristics including mobility, a well developed lysosomal enzyme system and the ability to degrade extracellular materials. Recently investigators at the University of Southern California have shown that

mononuclear phagocytes respond chemotactically to the products generated during bone resorption. The investigators at Washington University and the Jewish Hospital at St. Louis have extended these findings by identifying several purified constituents of bone matrix that cause the monocytes to migrate. They tested the chemotactic activity of three substances derived from bone matrix: 1) a collagen peptide from Type 1 collagen produced by mammalian collagenase; 2) A2HS glycoprotein, a plasma constituent that accumulates preferentially in bone and, 3)osteocalcin a peptide released during demineralization. All of the three components are powerful chemoattactrants for mononuclear phagocytes. They have further shown that the response to osteocalcin is mediated in part through a recepter on the monocytes.

One of the major features of inflammatory periodontal disease is the marked reduction in collagen in the periodontal tissues. According to previous studies this breakdown of the collagenous components in the tissues could be due in part to tissue collagenases elaborated by a variety of host cells in the periodontium, and in part to collagenases from oral bacteria. Investigators at the University of Connecticut have recently demonstrated that collagenase is produced by both *B. gingivalis* and *A. actinomycetemcomitans* , the pathogenic bacteria implicated in adult periodontitis and localized juvenile periodontitis, respectively.

About 50% of the epileptics on regular intake of phenytoin (PHT) respond to this drug by developing pathologic gingival overgrowth. Studies of such patients by investigators at the University of North Carolina, Chapel Hill, have shown that these gingival overgrowths of responder individuals contain specific kinds of fibroblasts. In cell cultures these fibroblasts are synthetically hyperactive; they produce increased amounts of protein and glycosaminoglycans, important components of connective tissue. Moreover, they are stable throughout numerous passages. However, the collagenase activity of responder cells is drastically reduced even though the amount of total collagenase produced is elevated. The increased amount of synthesis of protein and other components and the decreased degradation of collagen by collagenase may account for the characteristic enlargement of gingiva *in vivo* .

In another series of studies the investigators at the University of North Carolina have developed a cat model for the study of phenytoin-induced gingival overgrowth. By analyzing feline fecal samples, they have shown that about 63% of administered orally PHT remains unchanged. In cats, a major metabolite of PHT is phenytoin-glucuronide which is excreted in the urine. Preliminary studies show, however, that this compound is not a major component in the urine of phenytoin-treated human patients.

Meetings Sponsored

The NIDR Programs Advisory Committee, composed of the Subcommittees on Caries and Periodontal Diseases met once and the Subcommittee on Periodontal Diseases met twice during FY 1982. At the NIDR Programs Advisory Committee meeting Dr. Michael Cole and Dr. J. T. Hoffeld discussed the role of immunization in caries and periodontal diseases respectively. The results of Dr. Cole's studies suggest that oral immunization is a simple and effective method to induce specific secretory IgA which in turn impairs implantation and colonization of the cariogenic bacterium *S. mutans*. Dr. Hoffeld concluded that there is insufficient information concerning etiologic organism(s) and pathological process(es) to consider immunization against periodontal diseases as either a safe or an effective therapeutic measure at this time.

At the 4th and 5th meeting of the Subcommittee on Periodontal Diseases the Subcommittee evaluated and discussed the status of the Periodontal Diseases Program and the papers on different aspects of periodontal diseases for the NIDR Long Range Plan. The subcommittee also reviewed the progress reports of the three Specialized Clinical Research Centers for Periodontal Diseases and agreed that the quality of research at the Centers is good and that the Centers are productive.

The Proceedings of the May, 1981, NIDR Workshop on "Surgical Therapy for Periodontitis" was published in the *Journal of Periodontology* and a single copy of the reprint is available upon request from Dr. Samuel Kakehashi, Chief, Periodontal Diseases Program, Room 519 Westwood Building, National Institute of Dental Research, Bethesda, MD 20205. The proceedings of the symposium held in May 1981 in Buffalo on "Host-Bacterial Interactions in Periodontal Diseases" was published by the American Society for Microbiology and copies of the book are available from the publishers.

Future Plans

The Institute will continue its strong ongoing programs of basic research into the biologic processes underlying oral health and disease. These will include studies related to the identification and characterization of both pathogenic and nonpathogenic microflora in the oral cavity, studies related to immune mechanisms which

may result in periodontal destruction, and studies related to soft and hard tissue destruction.

Clinical studies have shown that removal of bacterial plaque and plaque products will arrest periodontal diseases. Both mechanical debridement and chemical deplaquing are of considerable value to keep the periodontal tissues in a healthy state. Preliminary studies indicate that it is feasible to develop effective antimicrobial agents and deliver them to the sites of infection. Accordingly, the program will encourage research to develop antimicrobials with a high potential for prevention and to develop effective delivery methods.

Since current methods of diagnosis are unable to recognize when the periodontal lesions are active, there is a need to develop accurate, rapid and objective methods to evaluate the disease and the effect of treatment procedures. Several methods are being tested for their value of detecting the active lesion. Continued efforts will be made to develop reliable and objective methods to measure the disease activity.

During the progression of destructive periodontal disease, bone loss occurs around teeth. Recent investigations suggest that certain treatment methods are conducive to the regeneration of periodontal support. There is also renewed interest in using transplant materials which can induce bone growth. The program will capitalize on these new avenues and encourage research activities in these areas.

Even though there is good reason to believe that the incidence of periodontal diseases is increasing in part because of the aging population in America, there is a dearth of well-designed epidemiological studies to determine accurately the incidence and prevalence of this disease. The Program would encourage studies to redress this need.

Summary of Research Highlights

Periodontal disease research highlights during FY 1982 included significant findings in clinical periodontology, in oral microbiology and immunology, and in basic studies on bone and connective tissue metabolism.

CLINICAL STUDIES

Three analytical methods, regression analysis, running median method and tolerance method were evaluated for their effectiveness in detecting periods of periodontal disease activity. The regression analysis is best suited to detect slow, steady changes but not for abrupt changes in attachment levels. The technique of running median is able to detect abrupt changes and the tolerance method has the greatest potential for early detection of changes in attachment level. The susceptibility of periodontal microflora to a large number of antibiotics was tested. In general penicillin was the most effective, followed by the tetracycline, minocycline and doxycycline. Erythromycin, carbenicillin, clindamycin and spiramycin, were not as effective as penicillin and tetracycline. Chloramphenicol and metronidazole were the least effective.

Minocycline is useful as an adjunct to scaling in the treatment of periodontal disease because of its effectiveness in suppressing the subgingival microflora. Systemic tetracycline suppresses *A. actinomycetemcomitans* and *Capnocytophaga* to negligable levels in all pockets. Several nonhalogenated salicylanilides have higher levels of antibacterial activity against a number of *Actinomycetes* than tribromsalan or fluorophene, two antimicrobial agents previously used in mouth rinses. Several of the newly branched alkylbisbiguanides appear to be potentially valuable agents in the control of periodontal microflora.

MICROBIOLOGY

A comprehensive study of microbial flora of experimental gingivitis show that *A. naeslundii, A. odontolyticus, F. nucleatum, Lactobacillus, S. anginosus, V. parvula* and *Treponema* species are the most likely etiologic agents of gingivitis. The greatest source of variation of total flora was observed from person to person. As gingivitis developed and progressed the flora became more diverse and complex. There was a sequential colonization of certain species of bacteria during the progression of the disease. Several new species *B. Loescheii, B. oris, B. briccae* have been identified and characterized. Some of new isolates of spirochetes have complex growth requirements. *T. denticola* has a right-handed helical configuration, while a number of other oral spirochetes are left-handed.

A. actinomycetemcomitans , the predominant pathogenic bacteria found in lesions of localized juvenile periodontitis occurs in three distinct serologic groups. The serum antibodies found against these organisms are serotype-specific. The new ELISA technique has made the identification and characterization of microorganisms less tedious and time consuming and will permit larger numbers of microbial samples to be analyzed.

IMMUNOLOGY

By using ELISA techniques it has been possible to identify antibody to several species of oral microorganisms in the crevicular fluid. Both *Bacteroides*

and *A. actinomycetemcomitans*, suspected pathogens in periodontal disease, produced soluble factors which inhibit chemotaxis of neutrophils. Nonpathogenic organisms such as *S. sanguis* and *S. mutans* do not inhibit neutrophil chemotaxis. Muramyl dipeptide, a product of bacteria, caused the macrophage to release more oxygen radicals, which facilitates efficient phagocytosis and the killing of microorganisms.

BONE AND COLLAGEN METABOLISM

Three substances — l)collagen peptide from Type 1 collagen; 2) 2HS glycoprotein and 3) osteocalcin peptide released during demineralization — are powerful chemoattractants for mononuclear phagocytes. Collagenase is produced by both *B. gingivalis* and *A. actinomycetemcomitans* , the suspected pathogens in adult periodontitis and localized juvenile periodontitis respectively. The responder fibroblasts isolated from gingival overgrowth of patients treated with phenytoin are synthetically hyperactive; they produce large amounts of collagen and glycosaminoglycans, important components of connective tissue. These cells also produce large amounts of collagenase but with decreased activity. In the cat model, 63% of PHT administered remains unchanged and is excreted in the feces. Studies of urine from cats and humans given phenytoin indicate that phenytoin-glucuronide is a major metabolite in cats, but it is not in humans.

CRANIOFACIAL ANOMALIES PROGRAM BRANCH

Introduction

The Craniofacial Anomalies Program Branch supports research and training in research related to the etiology, prevention, diagnosis, and treatment of craniofacial anomalies. Studies on basic mechanisms controlling craniofacial growth and development provide a foundation for understanding the cause and prevention of oral clefts and other congenital malformations. Clinical research is direacted at improving the treatment of these conditions. Basic and clinical research on craniofacial defects and disfigurement resulting from injury is also a major concern of the Program. A third area of activity involves malocclusion and related functional problems.

During FY 1982, the Program coordinated the preparation of State of the Science papers on the three program areas of Congenital Malformations, Acquired Defects, and Malocclusion. Summaries of these papers for use in the Institute's long-range plan have been prepared.

Administration

In FY 1982 a total of 7.6 million was awarded to support 72 research grants, which included 6 program projects, 55 regular research grants, 6 new investigator awards and 5 small grants. Two research contracts were awarded for $65,456, and the Program also awarded $778,996 to support 43 research trainees, including 30 fellows on 6 institutional training grants ($533,933), and 13 individual postdoctoral fellows. In addition, 4 research career development awards were made at a cost of $153,437 and 4 short term training grants were funded ($58,271).

Table 1 shows the distribution of grant support according to funding mechanism. Approximately 28% of the grant funds were used for program projects, and 56% for regular grants. Table 2 shows the distribution of research grants by scientific category. Compared to 1981, the overall level of funding in FY 1982 declined 3.5%, and the distribution of funds for specific categories within the Program showed only minor changes.

TABLE 1. FY 1982 RESEARCH AND TRAINING SUPPORT BY FUNDING MECHANISM

		No. of Grants			
		Active	Funded	Cost ($000s)	Percent
Program Projects	(P01)	6	6	$2,421	27.9
Regular Research Grants	(R01)	68	55	4,848	55.9
New Investigator Awards	(R23)	6	6	269	3.1
Small Grants	(R03)	5	5	74	0.9
Career Development Awards	(K04)	4	4	153	1.8
Institutional Training Grants	(T32)	6	6	534	6.2
Individual Fellowships	(F32 & F33)	13	13	245	2.8
Student Short-Term Grants	(T35)	4	4	58	0.7
Conference Grants	(R13)	1	-	-	-
Research Contracts		3	2	65	0.7
		116	101	$8,667	100.0

TABLE 2. FY 1982 ACTIVE RESEARCH GRANTS BY SCIENTIFIC CATEGORY

		Number	Cost	Percent
I.	Craniofacial Anomalies-General	27	$2,118	27.8
II.	Cleft Lip/Palate	18	1,846	24.3
III.	Other Congenital Anomalies	7	1,353	17.8
IV.	Malocclusion	28	2,062	27.1
V.	Acquired Defects	5	232	3.0
		85*	$7,611	100.0

* Includes P01, R01, R23 and R03

Staff Activities

During FY 1982, staff activities included visiting institutions, monitoring grants and contracts, communicating with researchers, participating in scientific meetings. Through these professional activities, staff was able to maintain close communication with scientists working in the field of craniofacial anomalies. These activities included:

A. Site Visits: Initial Review, Monitoring, Proigramming & Communication

2 T32 DE 07042-05, University of Indiana, Indianapolis	Feb 1982
2 RO1 DE 04517-04, St. Francis Xavier Hospital, Charleston, South Carolina	Apr 1982
2 PO1 DE 02872-14, University of Illinois, Chicago	Jun 1982
2 PO1 DE02848-13, University of Southern California, Los Angeles	Jul 1982
1 RO1 DE06412-01, University of the Pacific San Francisco	Aug 1982

B. Meetings

Symposium on Orthodontics and Bioengineering, sponsored by Grant No. 1 R13 DE05468-01, Dr. Charles J. Burstone, University of Connecticut, Hartford	Oct 1981
Annual Meeting of the International Association of Dental Research, New Orleans	Mar 1982
Annual Meeting of the American Cleft Palate Association, Denver	Apr 1982
Annual Meeting of the American Association of Orthodontics, Atlanta	May 1982

C. Sponsored Meetings and Seminars

Sponsored the Seminar, "The Incidence of Hospital-Treated Facial Injuries," presented by Mrs. Trudy Karlson, Bethesda	May 1982

Staff Development

Human Subjects Forum, sponsored by the Office of Protection of Human Subjects Against Research Risks, DRG, Bethesda	Feb 1982
Symposium on Clinical Genetics, Co-sponsored by the American Cleft Palate Association and the March of Dimes, Denver	Apr 1982
Step Module 5, sponsored by the Extramural Research and Training Program, Office of the Director, Bethesda	Apr 1982

Research Highlights

DEVELOPMENTAL BIOLOGY

The development of craniofacial structures involves embryonic cell migration and interaction. Cells originating in one area migrate long distances before differentiating into their final form. Tissues from dissimilar sources interact to influence one another's differentiation. For the cranial region, one of the most important cellular components consists of neural crest cells, which appear relatively late in the development of the neural folds at the time of neural tube fusion. Neural crest cells migrate extensively during development. This migration is temporally and spatially precise, and the cells eventually form such diverse derivatives as pigment cells, sensory and autonomic ganglia, teeth and connective tissue. Changes in the distribution and composition of the extracellular matrix including changes in the basement membrane and cell-associated proteins on the crest cells may trigger the onset of neural crest cell movement and exert some influence over the pattern and pathway of migration.

The role of the extracellular matrix and cell surface proteins in the control of neural crest cell migration is being studied in mutant and normal strains of mice by scientists at the University of California at Davis. The mutant mouse strains are being studied because their neural crest cell migration and differentiation are deficient. These deficiencies are expressed in the growing animal as a loss of pigmentation, an absence of sensory and autonomic ganglia and other cranial-facial lesions. A recent study of neural crest morphogenesis in normal mice revealed several interesting features of crest migration. Apparently the basement membrane is involved in the initiation of crest migration and also plays a role in directing crest migration by forming tissue barriers which the neural crest cells cannot penetrate.

These workers are now examining the basement membrane and the extracellular matrix in the region surrounding the dorsal neural tube at both normal and defective axial levels in the mice. Determining how the basement membrane may control cell invasiveness is important for understanding tumor growth as well as developmental anomalies.

Using the development of the ear as a model system, scientists at the University of Texas at Austin have studied the microanatomy of the tissue through which the crest cells must navigate to reach their targets. For example, in chick embryos, the development of the inner ear is initiated when neural crest cells, migrating in a predictable pathway under the ectoderm away from the neural tube, cause the overlying ectodermal cells to increase in height and crowd together. Soon, two oval discs of thickened ectoderm, closely adherent to neural crest cells, buckle and invaginate to form a round otocyst, the rudiment of the inner ear. As a result of analyses with the scanning electron microscope, these workers obtained evidence that cranial neural crest cell distribution may result from the ability of the migrating cells to follow an underlying mesodermal pattern. They showed that the path followed by the neural crest cell was predictable and in accordance with such a pattern. The mesodermal pattern is segmental, occurring as repeated circular domains along the midline termed somitomeres. Somitomeres are contiguous in the cranial region, but separate from one another in the caudal region as somites. For otic development, the particular neural crest cells migrate laterally under the surface epithelium in grooves in the mesoderm formed by abutment of somitomeres. A cranial population of cells utilizes the groove between somitomeres 5 and 6, whereas a caudal population invades the groove between somitomeres 7 and 8. Together, they encircle a region of the surface epithelium that develops into the otic placode. These investigations suggest that the precise distribution of cranial crest cells is the result of their interaction at a specific time with a patterned mesodermal layer whose surface is then modified by close apposition of surface ectoderm and the differential distribution of extracellular matrix. Disturbances in this highly complex developmental system apparently cause craniofacial anomalies.

The role of the extracellular matrix in craniofacial development is being investigated by scientists at Tufts University. They found that synthesis of hyaluronate, a major extracellular polysaccharide, correlates closely with cell migration and proliferation, and that hyaluronate degradation correlates with differentiation in several developing tissues and in repair and regeneration.

Previous studies indicated that interaction of hyaluronate with the surface of cells may have an important regulatory function in controlling cell movement, proliferation or differentiation.

In the past year, the investigators extracted, characterized and partially purified a protein which binds hyaluronate to the surface of cells and under certain conditions mediates the internalization of hyaluronate into the cell for the purpose of degradation. They also studied the assembly and regeneration of cellular coats of hyaluronate using drugs to manipulate these processes. In addition, a specific hyaluronate-binding probe is being developed to visualize hyaluronate in these cell coats as well as in extracellular matrices during various normal and pathological developmental processes.

Nerves influence a variety of developmental and regenerative phenomena and some evidence suggests that the pattern of nerve fiber growth may play a special role in the initial development of peripheral structures. Thus, early deficits in neural maturation may be causative factors in certain developmental deformities.

In research conducted at Case Western Reserve University, scientists are analyzing the role of the cranial nerves in the earliest stages of cranial development, and trying to determine how the trigeminal nerve is able to adapt to the rapid changes which occur during the development of the jaw apparatus. These studies are being carried out on the common frog, *Rana pipiens*, because the neuromuscular apparatus of its jaw undergoes dramatic reorganization during metamorphosis from the larval to the adult form.

These studies have revealed that the trigeminal neurons are among the earliest cells to be generated in the brain and to extend projections into the periphery. Only when these processes reach the branchial arch, do the target cells become organized into definite arrays that will give rise to the hard and soft tissues of the head.

It was also found that the trigeminal motoneurons become respecified during development. At metamorphosis the muscles that power the larval jaw degenerate and are replaced by newly formed muscles that move the adult jaw. However, both sets of muscles are innervated by the same motoneurons suggesting that the nerves may provide specific cues that cause the destruction of one set of muscles and the formation of a second set.

Although this investigation is geared to the maturation of the jaw apparatus in amphibians, it is also important to human facial development. In neonatal humans, the neural circuits controlling jaw function are set up for the stereotyped movements of suckling. Subsequently, their circuits are gradually replaced by the neural controls required for mastication and speech. In the frog there is an abrupt and dramatic remodeling of the oral apparatus; thus this animal provides a unique model for assessing neuronal reorganization in peripheral development.

It has been shown that excess vitamin-A in the culture medium can induce the formation of cilia in normally keratinizing epithelium and can also prevent cartilage formation. Investigators at the University of Maine are conducting studies to determine whether the ciliogenesis that is induced by vitamin-A is normal, and to determine the effects of excess vitamin-A on skeletogenesis in the mandible. Ultrastructural studies showed that excess vitamin-A in the medium of cultured embryonic chick mandibles does induce ciliogenesis within the epithelium. Normally this epithelium becomes a stratified squamous keratin-producing epithelium, but under the influence of excess vitamin-A, the epithelium became a thickened, simple cuboidal ciliated epithelium. Ciliogenesis occurred according to a pattern normal for developing tissues. Light microscopic studies showed that excess vitamin-A inhibits not only chondrogenesis but also genesis of membrane bone within the mesenchyme of cultured chick mandibles. Moreover, membrane bone formation proved to be more sensitive to the effects of vitamin-A than cartilage formation. Genesis of membrane bone was inhibited at lower concentrations of vitamin-A than was chondrogenesis. The mechanisms by which vitamin-A induces ciliogenesis within epithelia and prevents chondrogenesis and osteogenesis within mesenchyme are yet to be ascertained. This research is important because these effects are not conducive to normal development. Thus, an excess of this vitamin during development should be avoided.

CLEFT LIP AND PALATE

The development of the embryonic face involves several facial processes which are composed of mesenchymal cells and extracellular molecules including collagen, glycoprotein and glycosaminoglycans (GAGs). Failure of these processes to enlarge and/or fuse may result in a cleft of the lip or palate.

The role of hyaluronic acid (HA) in the morphogenesis of the embryonic mouse face has been studied by investigators at the University of the Pacific. It is generally believed that components of the extracellular matrix (ECM) that surrounds developing cells influence the ultimate behavior of these cells. In this study, mouse embryos were examined for HA and other components of the ECM during the period when the facial processes form and coalesce to form the definitive upper lip and primary palate. About one half of the substance synthesized in these areas at these times was HA. Thus, it appears that HA is synthesized in relatively large quantities and is a major component of the ECM at the time the facial processes are forming. Currently, the investigators are looking at the effect of removing HA from the ECM on the morphology of the mesenchymal cells in the facial region.

Physiological cell death occurs in selective locations in various embryonic organs at predetermined times during development. During development of the secondary palate, palatal medial-edge epithelium

undergoes selective degeneration, programmed to occur at a specific gestational age. This allows the two originally separate palatal processes to fuse and form the definitive secondary palate.

Investigators at Jefferson Medical College have demonstrated a correlation between increased levels of intracellular cAMP and palatal epithelial differentiation which terminates in death of these cells. Using radioimmunoassay, the investigators demonstrated that palatal cAMP levels rise during the period of palatal epithelial differentiation.

It has long been known that glucocorticoids administered in pharmacological doses to mice at mid-gestation results in cleft palate. In current studies, the Jefferson researchers have shown that maternal cortisone treatment also results in a dramatic depression of fetal palatal epithelial cAMP levels and, thus, may cause palatal clefting by altering epithelial differentiation. However, maternal cortisone treatment also inhibited palatal cAMP levels in mouse strains not susceptible to clefting. The data, therefore, suggest that cAMP is a key regulatory molecule controlling development of the secondary palate, but may not be specifically involved in clefting.

Although glucocorticoids, when administered to mice during mid-gestation result in cleft palate, the role of these steroid hormones in human clefting is not known. In mice, the susceptibility to steroid induced cleft palate is believed to be under genetic control.

One group of investigators at the University of Michigan has been trying to determine whether cortisone can induce cleft palate in man and if genetic factors play a role. Although they do not yet have an answer to this question, they have found that there are genetic differences in the physiological responses to glucocorticoids among different individuals and they can partially predict the glucocorticoid responsiveness on the basis of HLA typing. HLA refers to antigens found on human leukocytes. HLA typing has been of great importance in medicine because a number of diseases are known to be associated with certain HLA types. The Michigan investigators found that lymphocytes from individuals with certain HLA antigens are more sensitive to the effects of glucocorticoids than are lymphocytes from individuals with different HLA antigens. Although it is not yet clear whether this finding is related to cleft palate in man, HLA typing is of use in predicting which individuals are likely to suffer untoward effects (such as immunosuppression) from high levels of glucocorticoids.

Continuing studies of the genetics and biochemistry of the induction of cleft palate by glucocorticoids have also been carried out by researchers at the Children's Hospital of Philadelphia. These scientists have hypothesized that mouse strain differences in susceptibility to cortisone-induced cleft palate are related to differences in cortisone receptor proteins in the palate and that this phenomenon is controlled by genes originally thought to be at the H-2 locus, the so-called histocompatibility locus on the 17th chromosome. It has subsequently been shown that the locus for the major glucocorticoid receptor in the mouse is on the 18th chromosome. Therefore, the H-2 linked gene on the 17th chromosome either codes for a different receptor or has an indirect effect on the major receptor. These mouse studies give rise to the question as to whether potential susceptibility to drug-induced malformations in humans may be linked to HLA, the homologue of H-2 in man.

Other observations made by this group of researchers during the past year tend to lend further support to the hypothesis that glucocorticoids induce palatal clefting by blocking the release of arachidonic acid. Because arachidonic acid serves as the substrate for synthesis of both prostaglandin and thromboxane, the synthesis of these two substances is inhibited. One key observation supporting the hypothesis is that exogenous arachidonic acid given at the same time as dexamethasone (a form of cortisone) produces a significant reduction in the percent of fetuses with cleft palate. Experiments to show that arachidonic acid reduces the clefting action of cortisone have been done in sensitive strains of both mice and rats.

TREATMENT

An important hypothesis in the management of the speech problems of cleft palate children is that velopharyngeal competence (adequacy of the soft palate in closing the pharynx during speech) remains stable after palate surgery even though there may be changes in head size from growth and changes in pharyngeal size due to normal shrinkage of adenoidal tissues. Findings of one group of researchers at the University of Iowa supported that hypothesis for the majority of cleft palate children. However, there were exceptions. Children who had marginal palatal adequacy following the surgery developed increased nasalization of speech, apparently related to normal growth and normal adenoidal shrinkage. These findings, together with those from other research centers, emphasize how important it is to carefully follow children with marginal palatal adequacy even through the late adolescent years.

Cumulative findings from several studies by the same investigators, all in animals, continue to support the hypothesis that surgery for the cleft lip may be a more

important factor in facial growth than had previously been indicated. These findings are restricted thus far by the use of animals with surgically-produced clefts as the experimental model. However, the notion deserves further consideration because it may be helpful in determining the most appropriate timing for the separate procedures of lip repair and palate repair in order to optimize growth and speech development.

The relationship of airway obstruction to facial growth is a controversial topic which is presently the object of much discussion. A wide range of conditions, such as enlarged adenoids, allergies, and deviated septae, could lead to airway obstruction and, thus, could seriously affect facial growth. Therefore, it is believed that the clinical significance of this relationship is great.

Previously treated cleft lip and/or palate patients often present with a number of facial growth deviations, some of which are similar in form to those associated with airway obstruction. For example, the surgical procedure for pharyngeal flap, undertaken to improve hypernasal speech, creates a known increase in airway resistance (or relative obstruction) in cleft patients. To delineate the effects of partial airway obstruction on facial growth, investigators at the H.K. Cooper Clinic in Lancaster, Pennsylvania, are analyzing longitudinal growth data on patients who had pharyngeal flap procedures between ages 5 and 7. Facial growth patterns both before and after the flap surgery are being compared.

Presently, patients with cleft palate only, with unilateral cleft lip and palate, and with bilateral cleft lip and palate who had flap operations are being compared to patients of similar sex, cleft type, and facial dimensions and patterns, but who did not have pharyngeal flaps. Longitudinal data from the University of Michigan Center for Human Growth and Development is serving as non-cleft control data.

Preliminary findings indicate that when data are pooled with regard to cleft type, no consistent significant changes in facial growth occur as a result of the pharyngeal flap procedure. However, the cleft palate only group showed significant differences in several craniofacial growth dimensions occurring in the post-flap ages. In this group facial growth showed gradual progressive retrusions of both upper and lower jaws, and a steady increase in lower face height beyond that normally seen. All of these differences are changes in the expected direction, according to the hypotheses proposed for the effects of airway obstruction on facial growth. In addition, these data clearly indicate that pharyngeal flap surgery may cause very subtle, cleft-specific effects. Further work is currently in progress to more clearly define these differences.

OTHER CRANIOFACIAL ANOMALIES

Investigators at the Universitry of California (San Francisco) have been studying the function of certain craniofacial muscles in individuals with specific craniofacial anomalies. The largest group in this study are patients with hemifacial microsomia, and the next largest group consists of patients with premature fusion of cranial sutures (craniosynostosis). In the latter group, a major finding has been abnormal shortness of the temporal muscle. Patients with hemifacial microsomia develop neuromuscular patterns for some functions that differ from normal, because of structural resistance to certain movements and differences in the relationship of the mandible to its attached muscles and other structures. Some of these patients are being helped by treatment designed to enlarge underdeveloped muscles through exercise.

Other research by the same group of investigators is aimed at the study of bone formation by stimulation of muscle activity. The investigators use a model in which a bone graft is placed under the temporalis muscle and onto the skull of a rhesus monkey. To date, the findings indicate that muscle contraction at a rate higher than five contractions per second will inhibit bone remodeling in the graft. The duration of the interval between contractions necessary to induce bone formation remains to be determined.

Researchers at the University of Illinois have been investigating recurrence risks within families for hemifacial microsomia, a syndrome that involves defects of the ears, jaws, mouth, eyes, and occasionally, other structures. It had been generally accepted that there was no increased risk for future children after the birth of a child affected by this syndrome. This view may need to be modified as a result of a study involving 97 index cases whose pedigrees were analyzed carefully for occurrence and recurrence rates. It was found that eight percent (35/433) of the first degree relatives and six percent (11/176) of siblings had positive findings. The most frequent finding was that of minor ear malformations.

MALOCCLUSION

Tooth Movement And Bone Remodeling. The biological basis for orthodontic tooth movement consists of a remodeling response which takes place in the alveolar process as a result of applied stress. The characteristic histologic changes which occur include infiltration of phagocytic cells (macrophages and osteoclasts), resorption of the alveolar bone in areas of pressure and formation of new bone in areas of tension. The result of such tissue remodeling is relief of the stress and movement of the tooth to a new location.

The NIDR supports research directed at clarifying the basic mechanisms controlling bone remodeling and tooth movement. A thorough understanding of the molecular and cellular basis of tooth movement phenomena will lead to better control of clinical tooth movement and ultimately more efficient orthodontic treatment.

At the University of Connecticut, scientists are using clonal cell lines, obtained from an osteosarcoma, for the study of cellular aspects of bone differentiation. They found that one of the cell lines undergoes maturation in tissue culture. With increasing time and cell density, there is an enrichment in osteoblastic features such as cuboidal morphology, elevated alkaline phosphatase and sensitivity to bone-specific hormones. The rate of this process is subject to hormonal regulation. It is accelerated by hydrocortisone (or dexamethasone) and is retarded by hormones which increase the cyclic AMP level in the cell, for example, parathyroid hormone. Factors which affect maturation also affect growth, in an opposite direction. This is consistent with the known reciprocal relationship between growth and differentiation.

Vitamin D enhanced maturation in early cultures rich in immature cells, but inhibited maturation in the late cultures containing a large number of mature cells. This finding indicates that cellular responses to environmental stimuli depend on the state of differentiation of the responding cells, an observation consistent with previous reports by these investigators of mechanical and electrical effects on bone and cartilage cells. To better define the effects of environmental factors in these cells, the investigators grew them successfully in chemically defined media, where individual molecules could be studied alone or in combination. Under these conditions, physiological concentrations of vitamin D stimulated growth about three-fold and retarded maturation. This system provides an opportunity to examine under well defined conditions the concentrations and time-dependence of the effect of various agents on osteoblastic maturation.

At the University of the Pacific, investigators are studying changes in electrical potential in the periodontal ligament (PDL) in response to orthodontic forces applied to teeth. A 100gm orthodontic load applied to rat molars widened the PDL and created an area of tension. A tungsten microelectrode placed in such an area of tension recorded a drop of about 5-10mV in electrical potential. This reduced potential was maintained for as long as the load was applied, up to 20 minutes. Animals monitored after breathing stopped, due to anesthetic overdose, showed only a minimal response to loading after 120 minutes and no effect at all after 20 minutes. These results suggest that a sustained, electrical response to physiological loading is involved in the control of orthodontically-induced osteogenesis.

Scientists at the University of Pennsylvania are studying the optimal conditions for promoting alveolar bone remodeling through the intervention of electrical stimulation. Prior work showed that the rate of bone remodeling could be considerably enhanced through electrical stimulation. Orthodontic tooth movement, repair of bone loss due to periodontal disease, non-surgical bony closure of cleft palate, and prevention of mandibular alveolar bone loss in edentulous people are areas of potential clinical benefit.

In experiments in cats, electricity was supplied by miniature intraoral electronic power packs through electrodes placed on the gingiva adjacent to the teeth to which orthodontic forces are being applied. Since stationary electrodes used in earlier studies did not maintain their optimal position with respect to changing bone morphology, "tracking electrodes" which move with the teeth were developed. The orthodontic force magnitude remained constant (80gm), but the d.c. current provided by the 'tracking electrode' device varied from 5 to 30 microamperes. The rate of tooth movement in the cats was enhanced for teeth treated by the combined electric-orthodontic approach at all levels of current, but the best results were seen in the 10-20 microampere range. Below that range (5 microamperes) the effect of electricity on tooth movement was too subtle, and above that range (30 microamperes), gingival ulceration occurred near the anode.

The extent of the cellular response to electrical stimulation in cats *in vivo* was estimated by the use of immunohistochemical methods for the localization of cyclic nucleotides and prostaglandins, substances that mediate and modulate the effects of external stimuli on bone cells. The scientists found that the levels of cAMP, cGMP and PGE in alveolar bone periosteal osteoblasts increased, and that these increases occurred as early as 15 minutes after application of a constant d.c. current. These results indicate that the electric current penetrates rapidly through the gingiva to reach bone cells, and that the response of bone cells is mediated by substances known to be involved in the activation of cells by chemical messengers such as hormones, ions and drugs.

In other studies, technetium 99 methylene diphosphonate, a radioactive bone-seeking substance was used to detect sites of active bone remodeling. The investigators found that after 7 days of electric treatment the uptake of the radionucleotide was increased by an average of 59 percent in the bone

adjacent to the electrodes. Thus, they demonstrated that electric currents are capable of evoking enhanced bone remodeling in a localized area, and that this biologic response can be detected in the living animal.

The basic information gathered from these studies has provided sufficient background for human clinical trials which are currently being initiated with private funds.

ORTHOGNATHIC SURGERY.

Severe handicapping malocclusion affects approximately five percent of the population of the United States. Since these malocclusions are the result of severe disharmonies in the facial skeleton, they are difficult to treat by orthodontic procedures alone and usually require a combined surgical and orthodontic approach. A particularly difficult group to treat in this category are individuals with the "long face syndrome". As part of this condition these patients have excessive eruption of posterior teeth causing the lower jaw to be rotated downward and backward.

The causes of these severe skeletal dysplasias are being investigated at the University of North Carolina by clinical researchers who are studying the relationship between biting force, muscle function and tooth eruption in an attempt to understand the role of these factors in vertical facial growth. Their results show that long face adults have much less occlusal force than normal adults. In clenching, swallowing or in simulated chewing long face adults applied only half as much force to the teeth as normal adults. On the other hand, similar studies comparing normal and long face children between the ages of 6 and 11 showed no differences in occlusal forces. A comparison of the data for children and adults revealed that normal children exhibit about half as much occlusal force as normal adults, but long face adults have occlusal forces which are approximately the same, perhaps even slightly less, than normal or long face children. The data suggest that long face adults fail to gain strength in the jaw muscles during adolescence, while the jaw muscles of normal children are becoming much stronger. By adulthood, the difference is dramatic. From these data, it seems that the long face pattern can be recognized before dramatic differences in occlusal forces and, presumably, differences in strength of the jaw muscles, are evident.

Although these findings suggest that the decreased muscle strength may develop secondarily — perhaps in response to the abnormal skeletal relationship — the conclusion to be drawn is not clear. Muscular weakness could, in part, be a cause of the problem. Reduced biting force can allow increased eruption of the posterior teeth and hence contribute to the long face through downward displacement of the mandible. Further studies are being done to clarify these important findings.

There are several problems in maxillofacial or orthognathic surgery that need to be solved in order to improve surgical correction of gross malocclusion. Foremost among these is skeletal relapse, or the tendency of the altered skeletal segment to return toward its original position or form. For example, surgical correction of a small, or deficient lower jaw (mandible) often calls for a mandibular advancement, i.e. an osteotomy of the mandible and the pulling-forward of the fractured bone segment, in order to align the upper and lower jaws. Relapse may occur if the advanced mandibular segment moves backward to any degree at any time subsequent to surgery. Control of skeletal relapse by alleviating the factors responsible for it, therefore, is a major clinical problem. A second, related, problem concerns the growth of the face and jaws after mandibular advancement surgery. Although dogma has it that elective surgery should not be used to correct a developing malocclusion because the surgery itself will cause greater growth discrepancies, some surgeons believe such surgery in children is advisable and even desirable. Relapse in adult patients and growth restriction in young patients are, in large part, the same problem. That is, it is likely that the same factors that cause relapse in adults may cause growth restriction in children.

At the University of Michigan, research is being undertaken that involves simulation in monkeys of the surgery undertaken in humans. The short and long-term results of surgery involving mandibular advancement with muscles intact are being compared to results of advancement with detachment of the suprahyoid muscles. Long-term results are being assessed with serial radiographs of the head using radiopaque markers in the bone and muscles and by analysis of muscle function using electromyography. Short-term results are assessed by histologic analysis of the temporomandibular joint and by histochemical and biochemical analysis of muscle structure and function.

The results indicate that detachment of the suprahyoid muscles during mandibular advancement in adults has no detrimental effect on surgical result. Also muscle detachment does lead to greater short-term stability of the new jaw relationship. Preliminary results of mandibular advancement in juvenile monkeys, after less than one year, suggest that the monkeys in both experimental groups are growing normally.

ACQUIRED DEFECTS

Investigators at the Harvard School of Dental Medicine have studied the effects of condylectomy on

subsequent craniofacial growth as well as on regeneration of the condyle in monkeys. Eleven monkeys participated in the study. Five animals had a bilateral condylectomy and placement of an appliance to prevent the posterior and inferior collapse usually observed in the mandible after such surgery. Two animals had similar surgery but no appliances were placed. The appliance was placed in two monkeys who did not have condylectomy, and two animals had neither appliance placement nor condylectomy. During the nine-month study period, frontal and lateral radiographs, hand/wrist radiographs, and electromyographic and cineradiographic records were made. The findings of this study indicate that condylar removal results in severe growth inhibition of the entire face. Loss of growth was not resolved by placement of intermaxillary appliances. Hypertrophic cartilage was seen to form on the stump of the condylar neck. The time period for this study was not sufficient, however, to allow determination of ultimate shape and growth potential of the stump. These findings are of significance inasmuch as removal of mandibular condyles has been advocated in growing individuals with mandibular prognathism and in young patients with temporomandibular joint ankylosis or tumors.

Summary of Research Highlights

DEVELOPMENTAL BIOLOGY

Mutant strains of mice with neural crest abnormalities are being used to study the role of extracellular matrix and cell surface proteins in regulating neural crest cell proliferation, migration and differentiation. Recent studies have shown that the basement membrane played an important role in initiating and directing neural crest migration.

Studies of ear development in the chick have suggested that the precise distribution of cranial neural crest cells is determined by their interaction with a patterned mesoderm and also by the distribution of extracellular matrix.

Other research has shown that the synthesis of hyaluronate, a major extracellular polysaccharide, correlates with cell migration and proliferation and that the degradation of hyaluronate correlates with differentiation. Studies are now in progress on the mechanisms involved in the binding of hyaluronate to cell surfaces, its synthesis and degradation.

Studies in the frog, showed that at metamorphosis, jaw muscles degenerate and are replaced by muscles of the adult jaw. Both sets of muscles are innervated by the same motoneurons.

Studies of cell differentiation showed that excess vitamin A caused the formation of cuboidal ciliated cells in epithelium that is normally of the stratified squamous type. Vitamin-A also inhibited cartilage and membrane bone formation in the mandible of embryonic chicks.

CLEFT LIP AND PALATE

Studies in the mouse showed large amounts of hyaluronic acid during facial development. Research is underway on the effect of removing hyaluronic acid at different times.

Failure of the physiologic cell death that occurs normally in epithelial cells at the site of palatal fusion has been thought to be important in cleft formation. Research has shown a correlation between levels of intracellular cAMP and the palatal epithelial differentiation which terminates in the death of these cells. It was found that maternal administration of cortisone produced a dramatic reduction of fetal cAMP in both susceptible and nonsusceptible strains of mice.

Studies attempting to determine whether steroids can induce cleft palate in man have shown genetic differences in the physiologic response to glucocorticoids among humans. This variation appears to be related to HLA types.

Basic research in rodents suggested that glucocorticoids produce clefting in the mouse by blocking the release of arachidonic acid.

An important finding related to the speech of cleft palate children is the confirmation that successful surgical closure provides a functional result that is stable indefinitely, even though there are changes in head size from growth and changes in pharyngeal size due to the shrinkage of adenoids.

Cleft palate repair sometimes produces partial nasal obstruction. Studies of patients with cleft palate only showed growth changes after surgery believed to be secondary to reduced nasal air flow. Further research is expected to shed light on the effect of nasal obstruction on facial growth.

OTHER CRANIOFACIAL ANOMALIES

Clinical investigators have identified muscular deficits associated with hemifacial microsomia and premature fusion of cranial sutures. These defects have associated functional abnormalities which can be improved by specific exercise treatments designed to enlarge these underdeveloped muscles.

Bone remodeling in bone grafts placed under the temporalis muscle of rhesus monkeys was inhibited if

muscle contractions occurred at a rate of five or more per second.

Although it had been accepted that there was no increased risk for future children after birth of a child affected with hemifacial microsomia, recent genetic studies showed that eight percent of first degree relatives of affected individuals did have manifestation of the defect.

MALOCCLUSION

Research to clarify mechanisms of bone remodeling and orthodontic tooth movement showed that Vitamin D in physiologic levels stimulated growth three-fold and retarded maturation of bone forming cells in culture.

Studies of changes in electrical potential induced in the periodontal ligament by orthodontic forces indicated that a sustained, electrical response is involved in orthodontic tooth movement.

In other studies on alveolar bone remodeling with electrical stimulation, a properly applied electric current enhanced bone remodeling in a local area. Sufficient information has been gathered from these studies to justify human clinical trials.

Vertical dysplasias are among the most difficult dentofacial deformities to manage clinically. Recent research has shown that adults with "longface" syndrome are able to generate only about one half the biting force of normal subjects whereas affected children could bite like normal children. This finding is being investigated to determine if muscle strength or other factors are involved.

Relapse or return of skeletal segments toward their original position can be a serious problem for orthognathic surgeons. Lengthening the lower jaw has been among the procedures most likely to cause relapse in humans. In monkeys this type of surgery has so far produced short-term stability with no adverse affects when the suprahyoid muscles were detached. These animals are currently being followed to assess longer-term results.

ACQUIRED DEFECTS

Surgical removal of the mandibular condyle in monkeys caused severe growth inhibition which affected the entire face and was not prevented by placement in intraoral appliances. These findings suggest that the treatment of tumors, skeletal dysplasia or traumatic conditions in growing children should include removal of condyles only when no other recourse exists.

RESTORATIVE MATERIALS PROGRAM BRANCH

Introduction

The Restorative Materials Program serves as a primary focus at the NIDR for supporting research and development in dental biomaterials and instrumentation. Since the sequelae of oral diseases are damaged tissues, there is a continuing need for materials to repair these tissues and restore function and appearance.

Through grants, contracts and interagency agreements, the Program funds research in the development of new and improved materials. These research areas fall into eight categories: 1) restorative filling materials for repairing teeth; 2) bonding agents, adhesive coatings and cements to prevent decay on the chewing surfaces of teeth and at the margins of fillings; 3) intraoral prostheses for replacing missing teeth and other oral tissues; maxillofacial prostheses to replace defects resulting from congenital abnormalities, surgery, or accidents; 4) artificial tooth implants to replace missing teeth and to serve as anchors for bridges and dentures; 5) materials and techniques for improved root canal therapy; 6) transplants and replants of natural teeth; 7) diagnostic equipment and devices to improve dental care; 8) improved restorative materials for prevention.

Administrative

Table 1 shows the distribution of funds for research and research training in FY 1982. During FY 1982 the Restorative Materials Program Branch awarded a total of $3.7 million to support 52 grants and 3 interagency agreements for research on restorative filling materials, bonding agents, oral and facial prostheses, artificial tooth implants, endodontics, transplants/replants and general studies. These awards included 2 research career development awards. The program also awarded a total of $354 thousand to support 9 training grants and $40 thousand for 2 individual postdoctoral fellowships.

Table 1

FY 1982 Distribution of Funds

Restorative Materials Program Branch

	No. of Projects	Funds ($000s)	Percent
A. RESEARCH			
Grants			
Filling Materials	14	$ 758	16.9
Bonding Agents	4	229	5.1
Prostheses (Oral)	14	1136	25.3
Prostheses (Facial)	1	106	2.4
Implants	6	674	15.0
Transplants & Replants	2	108	2.4
General Studies	5	374	8.3
Prevention	1	66	1.5
Endodontics	3	178	4.0
Career Development Awards	2	78	1.7
Subtotals	52	$3707	82.5
Interagency Agreements			
Filling Materials	1 1/3	$ 434	9.7
Bonding Agents	1/3	151	3.4
Prostheses (Oral)	1/3	66	1.5
General Studies	1	135	3.0
Subtotals	3	$ 786	17.5
Totals for Research	55	$4493	100.0
B. TRAINING			
Institutional Grants	4	$ 303	88.3
Individual Fellowships	2	40	11.7
Short Term Training*	5	52	-
Subtotals	11	$ 395	100.0
GRAND TOTALS	66	$4,889	

*Multidisciplinary grants included here for administrative reasons only. Not included in percentages.

Staff Activities

During FY 1982 Program staff made visits to research institutions to program and monitor grants and contracts. Through these professional activities and participation in scientific meetings, they were able to stay abreast of scientific developments and to maintain close liaison with scientists working in the area of restorative materials. These professional activities are listed below:

A. Site Visits: Initial Review, Monitoring, Programming & Communication

University of Michigan, Ann Arbor	Oct 1981
National Bureau of Standards, Gaithersburg	Feb 1982
National Bureau of Standards, Gaithersburg	May 1982
National Bureau of Standards, Gaithersburg	Jul 1982
University of California, San Francisco	Sep 1982
Letterman Army Institute of Research, San Francisco	Sep 1982
National Bureau of Standards, Gaithersburg	Sep 1982

B. Scientific Meetings

Post-graduate Course on Amalgams, Ann Arbor	Oct 1981
Review Panel, AADR Fellowship Committee, Bethesda	Jan 1982
American Association for the Advancement of Science, D.C.	Jan 1982
American Association of Dental Research, New Orleans	Mar 1982
National Standards Committee MD 156, New Orleans	Mar 1982
14th International Biomaterials Symposium and 8th Annual Meeting of the Society of Biomaterials, Orlando	Apr 1982
Osseointegration of Clinical Dentistry Conference, Toronto	May 1982

C. Administrative

Randolph Macon Women's College, Lynchburg	Oct 1981
STEP Committee Meeting and Forum	Nov 1981
STEP Committee Meeting and Forum	Jan 1982
STEP Module #4, "Shrinking Research Dollars: Funding Issues, Mechanisms & Alternatives", Bethesda	Mar 1982
STEP Module #6, "Politics of Health: 1982, Bethesda	Mar 1982
White House Workshop, Washington, D.C.	Apr 1982
STEP Annual Planning Meeting, Harpers Ferry	Jun 1982
NIH Course, "Effective Communications", Harpers Ferry	Sep 1982

Research Highlights

ARTIFICIAL DENTAL IMPLANTS

After many years of laboratory and animal experimentation, research on artificial dental endosseous implants has moved into the human clinical trial stage. The past year's highlights include human clinical studies on porous titanium implants and on metallic blades; and laboratory and animal studies of implants coated with either porous high density polyethylene or porous polysulfone.

The investigation at the Medical University of South Carolina on artificial tooth roots is now in its 10th year. Last year's annual report cited experiments in dogs and monkeys to test the efficacy of a porous rooted titanium dental implant. These animal experiments indicated that two-stage implants of cylindrical design employing porous titanium offered the most potential for human usage. In the next phase of this research, long term clinical experiments were begun in Rhesus monkeys with implants supporting crowns and bridges. Eleven monkeys were employed in the study; 2 have been sacrificed and 9 are completing crown and bridge residence times of 36 months or more. Histological processing of the 4 implants in two monkeys is incomplete at this time. The 21 implants still functioning are asymptomatic and, although generally exhibiting chronic inflammation, are successfully being utilized in occlusion by the monkeys. The principal difficulty with the experiments at this point has been the necessity to replace cast gold bridges which have become badly worn as a result of heavy occlusion. Clinically, it does not appear that the heavy occlusion has had a detrimental effect on implant performance; however, final judgment as to its effects on the bone supporting the implant must await further study.

Because of the clinical success of the long term animal experiments and the favorable histologic results of previous monkey experimentation, limited human trials were begun. The first year's effort consisted of developing the necessary armamentarium and experimental techniques to perform human implantation, fabricating the implants, and selecting patients. Of over 400 patients evaluated, 15 were selected as having the best potential and 9 of these were entered into the program. Restorative and periodontal procedures have been completed on 8 of these patients to prepare them for implants. The first two patients were implanted during August and September, 1982. Seven other patients are currently being scheduled. The principal difficulty in the human experiments has been the amount of effort necessary to select and prepare patients for implantation. Although the first patients have had no pain or complications, modifications are being made in the procedure based on what has been learned during the first operations.

In another ongoing implant clinical study at Harvard University, 60 patients will be studied over a 5-year period. This project will utilize the blade implant to serve as the distal abutment for a four-unit fixed bridge. The implant-supported fixed bridge will be compared to a distally unsupported cantilever fixed bridge. These bridges are being placed on opposite sides of the lower jaw and are opposed by complete dentures. To date, 34 patients have been enrolled in this study. In 12 of these patients, implants and prostheses have been placed and the patients have been evaluated for at least nine months. Twenty-two additional patients are in the process of receiving their implants, bridges, and dentures. At periodic intervals patients are examined and given a prophylaxis if necessary.

The fixed bridges have been designed specifically for this study so that they can be removed by the investigators for periodontal evaluation of the supporting teeth and implants. Evaluation procedures include critical mobility measurements by periodontometry and supporting bone measurements using angle-standardized radiography. In addition, other clinical measurements are obtained, such as gingival and plaque index, amount of attached gingiva, clinical mobility, pocket depth, occlusion, and clinical complications.

In the annual report for FY 1980, we reported that investigators at the Medical University of South Carolina were evaluating endosseous implants made of a titanium core coated with a porous, high density polyethylene (PHDPE). This project has been continued at Emory University, where investigators have recently reported that the PHDPE-coated artificial tooth roots displayed a high incidence of failure when implanted in Rhesus monkeys. The tooth roots displayed increasing mobility soon after implantation. In most instances, failure was associated with mechanical breakdown of the polyethylene and with acute bacterial infection. In contrast, porous polysulfone-coated tooth roots of similar design displayed a low incidence of failure. Even when failing, the porous polysulfone implants displayed no mobility. The porous polysulfone performed like porous titanium implants of similar design being investigated in other studies.

These results indicate that porous polyethylene has insufficient strength to be used as a porous coating on artificial tooth roots. The fact that the implants underwent mechanical failure may be related to the relatively low tensile strength of the material, poor sintering of the porous coating, and/or biodegradation of the material. Because a higher percentage of these

porous polyethylene implants performed better in dogs than in monkeys, the results indicate that the Rhesus monkey is a more stringent model in which to test endosseous dental implants. The results also indicate that porous polysulfone is an efficacious attachment vehicle for dental implants, and can be used as a model material to test certain hypotheses about endosseous dental implants.

ADHESIVE BONDING

The development of successful resinous adhesives which would bond to human tooth structure has been an important research objective of the Institute and many biomaterials researchers for a long time. After approximately 25 years of continuous support for research in this field, it can be said that this objective has essentially been met. Materials and methods for bonding resins to both dentin and enamel are now available.

BIS-GMA resins, used in composites, sealants, and "bonding resin" formulations, are not inherently adhesive to enamel or dentin, but if the enamel is first subjected to etching with phosphoric acid, these formulations can be successfully bonded to enamel. Thus, during the past decade adhesive bonding to enamel has been highly successful in the practice of restorative and preventive dentistry and in the practice of orthodontics.

Obtaining adhesive bonding to dentin has presented a more complex problem. Acid etching on vital dentin is contraindicated because it affects the pulp adversely, and does not significantly aid bonding to dentin. However, a new procedure has been developed that can be used to prepare the surfaces of both dentin and enamel for bonding with composite resins. The new method requires an acidic mordant solution, a surface-active co-monomer,and a coupling agent. In this procedure the surface of the tooth is treated with an aqueous solution of ferric oxalate, a mordant chosen because it can dissolve residues left on the surface of the tooth and at the same time precipitate insoluble salts in the openings of the tubules, thus blocking these entrances to the sensitive pulp tissues. At the same time, this iron compound helps to bind the various other compounds to the dentin. Subsequently, the surface-active co-monomer NTG-GMA in acetone solution is applied and, after an acetone rinse, the coupling agent PMDM is applied. A commercial composite resin mix, containing reinforcing filler, is then pressed against this treated surface and allowed to harden.

Scanning electron microscopy showed that the iron oxalate solution alters the surface layers. The coupling agents then provide molecules which are bound to the surface and can polymerize with the resin of the composite material applied subsequently. The dentinal tubules are not enlarged or filled to any significant depth with the adhesive or polymeric materials.

In these tests with extracted teeth, the adhesive joints produced by the methods described above were tested for tensile strength after immersion in water at 23 degress for two to five days. With dentin, tensile adhesive bond strengths averaged 1,900 pounds per square inch. With enamel surfaces the average tensile adhesive strength was about the same as that obtained with the usual acid-etch technique, or 1,960 psi. Bonds with these characteristics are expected to be clinically useful. Durable adhesive bonding of composite materials could improve bonding of composite core materials to teeth for stabilization of crowns and bridges, treatments of cervical erosions, root caries, and other conditions by reducing the amount of dentin that must be cut for mechanical retention, thereby increasing patient comfort. Thus, these new findings are expected to have a significant beneficial impact on the day-to-day treatment of dental patients.

Another research approach to the development of dental adhesives has involved studies of natural products from marine animals. The ability of marine invertebrates such as barnacles, mussels and oysters to adhere to a variety of surfaces under water has long intrigued biomedical materials scientists. To gain insights into the mechanisms of this underwater adhesion process, researchers at the University of Connecticut Health Center have investigated the chemical properties of macromolecules in the adhesive secreted by the marine mussel *Mytilus edulis*. The characterization of the adhesive substance in the byssus of this mussel, as described in a recent publication, has enabled investigators in this laboratory to undertake a detailed analysis of the adhesive (the polyphenolic protein) with the aim of ultimately understanding the nature of its interaction with wet surfaces.

The most significant recent finding is that the polyphenolic protein appears to be largely a polymer containing a basic repeating oligopeptide sequence. Given that the molecular weights of the adhesive protein and oligopeptide are 130,000 and 5,000 respectively, one would assume that approximately 25 repetitions of the sequence occur in the protein. The sequence contains all of the 3,4-dihydroxyphenylalanine (DOPA), hydroxyproline, serine and threonine, and most of the lysine present in the polyphenolic protein. Despite the presence of hydroxyproline, the sequence does not appear to be collagenous. Studies are under way to sequence the oligopeptide.

Researchers at the University of Minnesota are also attempting to characterize the adhesive material secreted by the sea mussel, *Mytilus edulis.* They are attempting to determine the type of collagen contained in freshly secreted threads from mussels maintained in a laboratory sea acquarium. Byssal threads and bovine type I collagen (as a control) subjected to several laboratory procedures and the results compared. In contrast to the control collagen, the byssal threads produced only one high molecular weight band rather than the expected type I collagen products. The byssal threads also contained a low MW fraction of approximately 10,000 daltons, and this fraction corresponded to the protein residue after collagen is precipitated from a pepsin digest. The unusual stability of byssal threads appears to result from extensive crosslinking mediated by a low MW protein similar to the phenolic amino-acid-containing protein involved in the production of mussel adhesive.

From the results of this study and the previous one, it appears that the adhesive material from *Mytilus edulis* contains structural entities similar to but not quite identical to true collagen.

RESTORATIVE FILLING MATERIALS

Silver amalgam is one of the oldest and most popular filling materials used to restore teeth. Many investigators have made significant efforts to improve amalgam formulations in the hope that restorations placed with the new formulation would last longer. Presumably, less frequent replacement of defective fillings would reduce the trauma and cost to the patient, both in time and money and conserve professional resources. Most dentists have also made substantial efforts to increase the longevity of amalgam fillings by carefully following recommended procedures and many have advocated that all amalgam fillings be polished. Although more time is required to polish a metallic filling, it was believed that the smooth, highly polished surface would be less susceptible to deterioration.

During the past decade, long term controlled clinical studies have been conducted to determine whether the improvements in amalgam formulation and in clinical procedures had actually improved its clinical longevity. This research was conducted through an interagency agreement with the former U.S. Public Health Service, Division of Hospitals, and currently with the U.S. Army Institute of Research. The study was carried out in the first fully computerized dental clinical research facility in the country. Filling materials were placed under carefully controlled conditions in a patient population which would be available for long term annual re-examinations. The traditional parameters of clinical performance, such as deterioration of margins, wear, caries, tarnish and corrosion were evaluated without prejudicial knowledge of the materials being examined. The computer was used to maintain control of the patient population, keep track of the identity of the materials used to restore the teeth and the changes which occurred as time progressed, and most importantly, keep track of the reasons for replacement for each material.

The clinical behavior of two different amalgam alloys was examined over a ten year period. The two amalgam alloys, a spherical formulation and a dispersed phase amalgam alloy, differed significantly in regard to certain traditional laboratory values, such as degree of creep and content of gamma-2 phase, which are considered by some investigators to be predictors of clinical performance. The two alloys also differed in comparative rates of deterioration of the margins. Nevertheless, the longevity of the restorations, measured as the percent of restorations still functional after ten years, was found to be approximately 50 percent for both alloys. Therefore, in spite of differences found in the laboratory and in certain clinical parameters, there was no difference in the longevity of the two amalgam alloys at the end of ten years. In a companion study polished and unpolished amalgam restorations of three diversely different amalgam alloys were compard. After five years, the survival rate for polished and unpolished amalgams were not significantly different for any of the 3 formulations.

It would appear that in spite of manufacturers' claims to the contrary, changes in the formulation of dental amalgam alloys have had negligible effect on the longevity of dental restorations. Although it is possible to show statistically significant differences between various materials in regard to certain clinical parameters, such as marginal deterioration, these parameters are not reliable in predicting longevity. Similarly, the currently advocated procedure of polishing amalgam restorations has only questionable benefits in regard to increasing a restoration's longevity.

Although significant advances have been made in the formulation and production of amalgam alloys during the past two decades, these advances have apparently not been reflected in actual clinical performance. Thus, one is led to the conclusion that the longevity of amalgam restorations is influenced more by extraneous environmental and human manipulative factors than by changes in composition and formulation. Moreover, costly research efforts to develop a reliable laboratory or clinical predictor of longevity have not been successful. Consequently, future research should concentrate on studies to assess environmental and

human manipulative factors which influence these restorations.

Investigators at the Medical College of Georgia have studied the fate of dental caries under a plastic sealant. Previously, the investigators had confirmed that carious lesions become sterile and do not progress when sealed off by the plastic coating from the nutrients in the oral environment.

The specific aim of their current clinical research is to evaluate a filled sealant as a restorative material for small occlusal (Class I) carious lesions without cavity preparation, using procedures developed in a previous research project. These procedures consist of a technique for applying a filled-resin/sealant system as a conservative restorative, and a technique for placing a combined amalgam/sealant restoration. Approximately 150 patients each with two similar Class I lesions will be treated. Half the lesions will be restored with a quartz-filled resin/sealant system and the other half with a higher copper amalgam. Seventy-five of the amalgam restorations will be placed in a conventional manner and 75 amalgam restorations will be more conservative, with all adjacent and remote pits and fissures sealed by the plastic coating before placing the amalgam. The efficacy of such treatments will be assessed at 0, 6, 18 and 36 months by clinical evaluation using Ryge's criteria, Mahler's photographic criteria, and by using standardized radiographic procedures. If the filled sealant restoration is judged clinically equivalent in anatomic form and in marginal integrity to the amalgam restoration and radiographically acceptable after 3 years, no further treatment may be required.

The proposed research is designed to determine the feasibility and efficacy of such a restorative treatment program. Early indications are that both of these new conservative methods may offer significant improvements in the performance of occlusal restorations and in the conservation of tooth structure.

In a laboratory project at the Medical University of South Carolina, scientists have investigated the properties of composite dental filling materials. Extensive compressive fatigue data have been generated to compare two commercial products: a glass filled and a quartz filled composite. These formulations have very similar polymer phases, filler particle size distributions and filler volume fraction so that the most significant difference is particle composition. Previous clinical studies have indicated that the glass filled product is more durable than the quartz filled material and that the two products show different modes of degradation. The scientists hope that appropriate laboratory experiments will lead to an explanation of these phenomena.

The compressive "fatigue limit" for each material was determined at 500, 1000, 5000, 10000, 50000, and 100,000 stress cycles with specimens immersed in 37°C water. Short term testing has been completed and long term testing (100,000 cycles) at 22°C. under both dry and wet conditions has been partially completed. The most significant observation of these studies is that fatigue and failure seem to be a function of water absorption.

At high stresses (low number of cycles) the fatigue life of the glass filled product exceeded that of the quartz filled product under all test conditions. However, at greater than 10,000 cycles, the fatigue strength of the glass filled product in 37°C. water was less than that of the quartz filled product. This crossover in fatigue curves suggests that at low cyclic stresses, different failure processes are active in the two materials.

Water absorption studies have provided insight into the nature of the failure processes. These studies indicate that the glass filled material absorbs more water and at a higher rate than does the quartz filled material. The quartz material reaches an apparent equilibrium water content, but the glass filled material continues to take up water. Presumably, this continued uptake of water accounts for the decline in fatigue strength as the cycling frequency is increased.

The clinical significance of the work is that the laboratory fatigue and water absorption studies show distinct differences between the glass filled and quartz filled materials. Thus these findings can be correlated with the clinical observations that the degradation modes of the two materials are different.

Assessment of the comparative clinical performance of different dental filling materials depends upon the ability to detect subtle changes. Because of varied environmental or other reasons, the degree of change in different individuals with only one filling material over a given observation period can span a wide spectrum. For example, some patients have restorations with no wear or barely detectable wear, whereas others have restorations of the same material and age which show easily detectable loss of material. If the objective of the study is to compare the wear rates of several filling materials, each with its own spectrum of wear, the need to perceive and rank the changes becomes a formidable task.

One technique which has been employed to solve this dilemma is to take impressions and make replicas of the teeth with the fillings of interest. One of the many

advantages of this procedure is that each replication be coded so that the identity of the material is not disclosed and subsequent assessments may be made in an unbiased manner and under the same conditions of observation. The next task of the experiment is to rank the replicas from the worst to best. If indeed there is a real difference between the wear rates of the different materials, it can be expected that certain materials, in spite of their own individual spectrum of wear, congregate at either the better or worse end of the overall rankings. Appropriate statistical tests can then be performed upon the rankings to confirm or deny the statistical significance of the differences noted.

The procedure described is comparatively easy when the replicas are few in number. However, in a large clinical study, where the number of replicas can be in the hundreds, sorting them into an orderly sequence is at best difficult, time consuming, and mentally and physically fatiguing. To solve this problem, investigators working under an interagency agreement with the U.S. Army Institute of Research prepared a computer program.

In ranking the coded replicas into their correct order of increasing severity, the dental evaluator examines only one replica at a time and decides whether it is better, worse than, or equal to the indicator replica. The computer records each new decision and reorders all the replicas into their relative ranking automatically. This program reduces the number of decisions by the human evaluator to an absolute minimum.

The program has been utilized to compare the wear rate of an experimental strontium composite resin with a proprietary composite resin. The manufacturer of the proprietary composite had claimed that the two composites did not differ in rates of wear. However, analysis of the rankings revealed that the proprietary material exhibited greater wear than the experimental strontium composite.

PROSTHETIC MATERIALS

Investigators at the University of Georgia are continuing studies to develop new, lower cost alloys suitable for bonding with porcelain. Porcelain bonded to metal has become very popular as a restorative dental material because it not only provides strength and durability, but also provides excellent esthetics. This type of appliance has been used for almost twenty-five years with varying degrees of success. Typically, the early alloys consisted of 95% gold. During the nineteen seventies, however, the need for gold replacement metals became urgent. This search still continues. One of the characteristics sought in such a metal is the ability to form a surface oxide, because oxide formation is thought to be necessary for bonding with porcelain. Systems currently in use for these purposes include the silver-palladium alloys, which contain a few percent tin and/or indium to form the oxide layer. Nickel-chromium and gold-palladium-silver systems have also been used. With the silver-palladium system, the porcelain may develop green discoloration from vaporization of silver from the metal surface.

In one of the recent studies, it was learned that on the silver-palladium alloy surface, mushroom-like nodules develop during the heating process rather than a uniform layer of external oxide. On further examination internally, it was found that oxidation had occurred, but as internal oxidation rather than external oxidation. Thus, the porcelain was being retained on this type of alloy not by an oxide-bonding layer, but rather by mechanical interlocking with the mushroom-like nodules. This mechanical retention, although it has appeared to be adequate, is theoretically less desirable than having a chemical bonding through the oxide layer, because the mechanical retention could permit ingress of fluids and consequent discoloration under the porcelain. In contrast, a uniform oxide layer would act as a barrier to vaporization of the silver and, hence, could very well prevent the greening problem in this type alloy.

Therefore, the investigators are looking for other additives to the silver-palladium system which will promote external oxidation rather than internal oxidation, and also would reduce the amount of precious metal, thus providing economic as well as scientific gains. Substituting for the expensive metals and achieving a better esthetic characteristic, as well as providing a good bond, is their goal. It is anticipated that such an alloy will be developed and tested in the future. Such an alloy would provide the dental profession with a less expensive, yet superior alloy for porcelain and metal bonding.

RADIOLOGY

Investigators at the Medical College of Georgia are investigating ways to increase the value of dental x-rays. In setting up radiographic reference guide sets, radiographs of lesions with known depths were duplicated. It was observed that radiographic details could be enhanced by varying the exposure time during the duplication procedure. A review of the literature and a consultation with investigators at Vanderbilt University School of Medicine revealed that little work had been done on the possibilities of obtaining improved detail by duplicating dental radiographs. Such an approach would not only improve diagnosis, but would also decrease radiation exposure to the patient, since radiographs without satisfactory detail would simply be

duplicated to bring out the detail, and would not have to be retaken. The investigators hope to develop a device that would instantly provide duplication of a single radiograph at various intensities; such a device would increase the diagnostic capabilities of the dentist. Collaboration with an investigator at NIDR revealed that a fair amount of the theoretical basis for this approach has already been developed. The investigators are planning to use a G.E. video system and Logatronics computer-processing system in their future attempts to obtain contrast enhancement appropriate for dental applications. At present, the promise of this approach for practical application in dentistry remains uncertain.

Future Plans

Staff anticipates an increase in applications for tooth implant research. A program announcement encouraging applications on clinical studies, animal model studies, mechanical function, and design factors (shape, size, material and surface texture) has appeared in the NIH Guide to Grants and Contracts. The expected increase in activity should generate valuable data in this important field.

In collaboration with the Intramural Program's Diagnostic Methodology Section, the Program will continue to support research at the National Bureau of Standards to develop a radiographic system which is expected to improve the detection of dental pathologies and document the progress of treatment without over-burdening the patient with radiation. A multi-position x-ray source and an x-ray detector will be developed which can produce 8 to 30 images in approximately one second, each image being taken at a slightly different projection geometry with respect to the teeth being investigated. This system will be specifically designed to interface with an image processing computer to be used for experimental research by the Diagnostic Methodology Section. It is anticipated that ultimately clinicians will be able to observe and record the progression of dental disorders, such as caries and periodontal disease, more accurately.

In an Interagency Agreement with the Letterman Army Institute of Research in San Francisco, work will continue on the evaluation of the long-term performance of restorative materials. This research program has access to over 1,000 patients with approximately 5,400 restorations. This data base has clinical information on amalgams, anterior and posterior composites, and porcelain-fused-to-metal restorations. Certain of these restorative materials were placed under controlled clinical conditions more than 14 years ago. Approximately 66% of all restorations are still in place and the majority have been evaluated for longer than 5 years.

In addition, the study of the incidence of nickel sensitivity in dental patients will continue. To date,they have conducted dermal patch tests on 439 patients. For valid statistical analysis they must test a larger number of patients, including some individuals with documented intraoral nickel-containing dental appliances.

Program staff are preparing for the publication of state-of-the-science review papers covering eight areas: amalgams, composites, cements, maxillofacial prostheses, oral prostheses, endodontics, implants, transplants, and replants. These papers will be published as a monograph through the Federation Dentaire Internationale,Commission on Dental Products.

Summary of Research Highlights

ARTIFICIAL DENTAL IMPLANTS

Research on implants included human studies on porous titanium implants and on metallic blades; and laboratory animal studies on implants coated with porous polyethylene or porous polysulfone. The titanium implants, two-stage cylindrical devices, were studied in monkeys and are now being tested in humans. In the monkey experiments implants have functioned successfully for long periods and remained asymptomatic, except for gingival inflammation. In the human trials 15 patients have been selected, 8 patients have been prepared for implants and 2 patients have received implants.

In the study of blade implants, 60 patients will be followed for 5 years. Of 34 patients enrolled so far, 12 have received implants and 22 are undergoing preparatory treatment. Comprehensive evaluation including mobility measurements, alveolar bone measurement, and other periodontal assessments will be performed. In the third study, endosseous implants coated with polyethylene failed in rhesus monkeys because of mechanical breakdown associated with acute bacterial infections, whereas porous polysulfone-coated implants of similar design had a low incidence of failure.

ADHESIVE BONDING

The BIS-GMA resins have been successful in composite fillings, sealants and orthodontic bracket attachment, but they do not adhere to dentin. This past year saw the development of a new procedure to prepare the surfaces of both dentin and enamel for bonding with composite resins. The new method

involves the application to the tooth surface of ferric oxalate (an acidic mordant), a surface-active co-monomer (NTG-GMA) and a composite resin mix. These treatments block the dentinal tubules, and make it possible to achieve clinically suitable bonding strengths.

The underwater adhesive substances secreted by sea mussels contain a polyphenolic protein of 130,000 daltons, which has structural entities similar to but not identical to those of collagen.

RESTORATIVE FILLING MATERIALS

The clinical behavior of two amalgam alloys was examined in a computerized dental clinical research facility. The two amalgams differed in certain laboratory values, such as creep and content of gamma-2 phase, which are believed to predict clinical performance, and also differed clinically in marginal deterioration. Nevertheless, longevity, measured as the percent of restorations still functional after ten years, was 50 percent for both. In a companion study, polished and unpolished amalgam restorations did not differ in five-year survival rate. In view of these findings, future research should assess environmental and operator factors which influence these restorations. In another project, two conservative procedures for small occlusal carious lesions are being tested. In one, a filled resin sealant is applied without cavity preparation, and in the second a combined amalgam/sealant restoration is placed, with the sealant applied before the amalgam.

A laboratory project is comparing a glass-filled with a quartz-filled composite. It was known that the glass-filled product was more durable, and had a different degradation pattern. Tests at high stresses confirmed that the fatigue life of the glass-filled composite was greater than the quartz product, but at low cyclic stresses, the strength of the glass-filled product was less than the quartz. Subsequent studies indicated that these differences were related to differences in water absorption.

Investigators recently designed a computer program to aid them in making rapid assessments of the clinical wear of restorations. After different intervals, replicas of the teeth are made and coded, and then are ranked in order of increasing wear by a dental evaluator who compares only two replicas at a time. He decides whether one is better, worse, or equal to the indicator replica. The computer records each new decision and reorders all the replicas into the correct ranking. This system was recently used to show that the wear rate of a proprietary resin was greater than the manufacturer claimed.

PROSTHETIC MATERIALS

In studies to develop low-cost alloys for bonding with procelain, investigators found that a silver-palladium alloy develops nodules on its surface, rather than an external oxide layer, which would presumably provide chemical bonding. The nodules provide only mechanical interlocking, which may permit the ingress of fluids and cause discoloration. Therefore, the scientists are seeking additives which promote bonding.

RADIOLOGY

In recent studies to improve dental X-rays, radiographic detail was enhanced by varying the exposure time during duplication. This observation is being exploited in an attempt to systematically improve detail without subjecting patients to additional radiation from retakes.

SOFT TISSUE STOMATOLOGY & NUTRITION PROGRAM BRANCH

Introduction

The Soft Tissue Stomatology and Nutrition Program Branch supports research in four major areas: oral soft tissue diseases, nutrition, salivary glands and their secretions, and mineralization. The program's main objectives are to obtain knowledge of a) the etiology, diagnosis, treatment, and prevention of oral soft tissue diseases and disorders, b) the role of nutrition in the growth, maintenance, function and health of hard and soft tissues of the craniofacial complex, c) the development and function of normal and abnormal salivary glands and their secretions, and d) the mechanism(s) of mineralization, with special emphasis on the cells and regulatory systems which affect the structure, function and repair of bones and teeth.

Administration

During FY 1982, the program awarded 79 research grants at a cost of $6,485,442 and one conference grant at a cost of $5,000. Table 1 illustrates the distribution of grant funds by subject category.

During this fiscal year, 4 institutional training grants received $241,531 for the support of 9 postdoctoral trainees. Funds were also provided for 6 individual postdoctoral fellowships ($105,312), one senior fellowship ($25,000), 5 career development awards ($186,030) and one career award ($32,670).

During FY 1982, a Program staff member represented NIDR on the NIH Nutrition Coordinating Committee and served as chairperson of its Subcommittee on Nutrition Education. These activities involved the presentation of verbal and written accounts of NIDR's nutrition research projects. Program staff also represented the NIDR on the NIH Digestive Diseases Coordinating Committee and on the NIH Cystic Fibrosis Coordinating Committee.

During this fiscal year, the two contracts jointly sponsored by NIDR and the National Institute of Allergy and Infectious Diseases to determine the clinical efficacy of the antiviral compounds were discontinued. The interagency agreement with the Veterans Administration Hospital in East Orange, New Jersey to improve the detection of early squamous cell carcinomas of the oral cavity was completed. The principal investigator is now receiving funds from the National Cancer Institute to continue studies of the role of alcohol and smoking in the etiology of oral cancer, a project which was started with NIDR funding under an interagency agreement. Two contracts to study various immunological responses of human patients with aphthous ulcers were also concluded.

To set the stage for the development of an NIDR long range plan, consultants expert in each major scientific area supported by the program prepared state-of-the-science papers. Staff has now reduced these to abstract form as the next step in preparing the long range plan.

TABLE I. DISTRIBUTION OF ACTIVE GRANTS FOR FY 1982

	Projects	Funds ($000's)	Percent
RESEARCH GRANTS			
Nutrition	6	$ 537	8.0
Salivary Secretions	21	1,648	24.6
Soft Tissue	25	1,707	25.5
Mineralization	33	2,807	41.9
Totals	85	$6,699	100.0
TRAINING			
Individual Fellows	7	$ 129	34.8
Institutional Training Grants	4	242	65.2
Totals	11	$ 370	100.0
GRAND TOTALS	96	$7,069	

STAFF ACTIVITIES

Monitoring, Evaluating and Programming Visits

1. University of North Carolina, Chapel Hill
   October 13-14, 1981 (Project Site Visit)

2. Eastern Virginia Medical College, Norfolk
   May 16-17, 1982 (Programming Visit)

Scientific Meetings

1. American Society for Microbiology, Atlanta
   March 8-12, 1982

2. AADR Meeting, New Orleans
   March 17-21, 1982

3. American Association of Orthodontists, Atlanta
   May 3-5, 1982

4. American Society for Virology, Ithaca
   August 2-5, 1982

Research Highlights

SALIVARY GLANDS

Investigators at the University of Connecticut have demonstrated that the acidic proline-rich proteins (PRP) are natural constituents of formed enamel pellicle. In more recent investigations they have collected pellicle on enamel slabs attached to orthodontic wires in patients with high and low plaque scores and high and low gingival and caries indices. The results indicate that PRP formed in periods from 1 to 24 hours in caries-free and plaque-free individuals accounts for a higher percent of total pellicle protein than that found in subjects with high indices for plaque, gingivitis and caries. Thus, high acidic PRP levels are associated with low rates of plaque formation.

In related studies the University of Connecticut investigators have perfected an indirect immuno-ferritin technique for electron microscopic detection of the acidic proline-rich proteins (PRP) in the parotid and submandibular glands of Macaca fascicularis. This technique enabled the investigators to identify PRP in specific cellular granules and in specific Golgi transfer vesicles and in vesicles budding from the Golgi apparatus. The data suggests that the proline-rich proteins are synthesized and packaged by conventional exocrine mechanisms and that they are transferred to the secretory granule of the parotid and submandibular gland of the Macaca fascicularis as discrete aggregates which remain as a separate "cap" area in the granule. In future studies the PRP can serve as a marker for exocrine secretion from these organs.

New evidence from investigators at the Medical College of Georgia indicates that fluoride ingested after teeth have erupted is cariostatic. The immediate source of fluoride causing this topical effect could be the drinking water itself, gingival crevice fluid or saliva. In a previous report it was noted that the level of fluoride in the gingival crevice fluid of the dog is nearly identical to the plasma fluoride level. Recently, the Georgia investigators have extended their investigations to studies of parotid and submandibular salivary fluoride concentrations and attempted to relate them to the amounts of fluoride ingested and to plasma fluoride levels. In this study the fluoride concentrations of plasma and of parotid and submandibular ductal saliva from 5 fasting human adults age 24 through 40 were compared to corresponding concentrations measured 2 hours after ingesting 22 mgs. of sodium fluoride in gelatin capsules. After ingestion, peak plasma fluoride levels were reached in one hour. The data indicate that both parotid and submandibular ductal saliva change simultaneously and proportionately with those of plasma fluoride. Parotid saliva fluoride concentrations were 70 to 90% of plasma levels, and submandibular saliva fluoride levels were even closer to plasma levels. Since the fluoride concentrations available to the teeth from saliva and gingival crevice fluid approach those of plasma, the cariostatic effect may be derived from these systemic sources as well as from the topical effect of drinking water on erupted teeth.

SOFT TISSUES

An investigator at the University of Iowa has established an *in vitro* method for measuring the permeability of various keratinized epithelia. The new method uses porcine tissue because it is similar in structure to that of humans. Measurements of horseradish peroxidase and water transport established that epidermis was the least permeable, gingival epithelium more permeable and sub-linguial epithelium the most permeable of those tissues examined. Information on the permeability of human oral mucosa is important since oral mucosa is exposed to foods, beverages, medicinal, mechanical and bacterial irritants. In addition, drugs can be designed to take advantage of the selective permeability of these epithelia for specialized administration. The Iowa investigator proposes that the permeability barrier of these epithelia consists of lipids or glycolipids rather than phospholipids as has been suggested previously.

Another investigator at the University of Iowa is concerned with the growth, development and hyperplasia of oral epithelium. It was shown that the rates of epidermal cell proliferation are related to cell synthetic activities in the basal and suprabasal strata. The balance between rates of cell formation, maturation and death determine the condition of the normal tissue. To help understand the balance, the investigator used such pharmacologic agents as epinephrine, isoproterenol, norepinephrine and dibutyryl cyclic AMP to alter glycolysis and amino acid incorporation into normal epidermis. The results suggest that adrenergic agents and cAMP cause a reduction in epidermal metabolic activity and cell proliferation, whereas increased rates of proliferation were associated with epidermal loss of beta adrenergic responsiveness.

In work at the State University of New York, Stoney Brook, keratinocytes from an invasive squamous cell carcinoma of the floor of the mouth have been maintained in culture for long periods. These cultured cells have a lifespan of 30-40 doublings, cannot stratify, but can serve as the hosts for a lytic infection by adenovirus type 2, which normally only infects suprabasal cells. These malignant keratinocytes have thus differentiated to a point where the adenovirus type 2 lytic replication can take place, but not to the point where stratification can occur. When the keratin from

these malignant keratinocytes was separated and purified, a previously unknown protein with a molecular weight of 40,000 daltons was present, but the 58,000 dalton molecular weight keratin usually found in normal keratinocytes was absent. Thus, it appears that the regulation of the production of this keratin in the malignant cells is altered. In related research this same investigator has succeeded in maintaining the viability of human papillomavirus (HPV) through several passages in culture for at least three weeks. He found that the human papillomavirus DNA was not integrated into the host cell genome but remained as a stable episome in the host cell. Since this finding is unusual, the scientist will continue his efforts to determine the mechanism of maintenance of the episome and to find out how the episome alters normal cell function.

MINERALIZATION

Investigators at Yale University have developed a model for studying cellular events in bone remodeling. The model utilizes the buccal surface of the mandibular alveolar ridge of the rat. When opposing maxillary molar teeth are extracted, the mandibular teeth erupt; these events induce a wave of bone resorption along the entire segment of the affected mandible. The resorption phase is followed by a reversal phase and subsequent bone formation phase. Each phase is characterized by a reproducible sequence of activity of different cell types. After induction, bone resorption was evident within three days, peaked in 4-5 days and then decreased sharply. The cells responsible for the initial resorption were mononuclear phagocyte-like cells which initially reached the bone surface by cytoplasmic extensions between osteoblasts. The plasma membrane of these cells next to the bone surface first developed invaginations (coated pits) and then the typical ruffled border of the osteoclast became evident. Multinucleation became evident by the fourth and fifth days. At the beginning of the reversal stage (6-7 days), osteoclasts detached from the bone surface and resorption cavities (Howships lacunae) became lined by a second wave of mononuclear phagocyte-like cells, some of which became loaded with crystalline material. A dense granular collagen-free layer was seen near the end of the reversal phase (7-10 days) on the bone surface; this granular layer calcified, forming the cement line. During this period, cells with the morphological characteristics of preosteoblasts and osteoblasts appeared within the lacunae. Newly synthesized osteoid was then deposited against the cement line (10 days), thus marking the beginning of an active bone formation phase.

In related studies on tooth eruption an investigator at the University of Massachusetts has observed the infiltration of the coronal aspect of the tooth follicle by mononuclear cells just prior to the onset of massive resorption of the adjacent crypt and deciduous tooth roots. This observation is compatible with the hypothesis that osteoclasts originate from mononuclear cells. Additional studies will be done to determine if these cells fuse to form multinucleated osteoclasts or perform some other function.

Investigators at Children's Hospital Medical Center in Boston have made considerable progress in understanding the role in bone metabolism of the protein which contains the amino acid gamma-carboxyglutamic acid (Gla). This protein with a molecular weight of 6,000 daltons was first isolated by this group and named osteocalcin, because it presumably binds calcium through its Gla residues. It has been found that this vitamin K-dependent protein is synthesized in bone and is derived from a high molecular weight (70,000 dalton) precursor. Unlike other Gla containing proteins, it is not synthesized in hepatic tissue. In studies of bone fractions of different density from normal animals, it was discovered that the Gla to calcium ratio was constant throughout all stages of bone formation and was independent of animal age. However, G1a levels in the poorly mineralized portion (low density) of rachitic bone were elevated 20 fold over the Gla levels in control bones and the dense fraction of rachitic bones showed almost the same elevation in Gla concentration.

In related studies a survey of the Gla content of ectopic calcifications indicated that Gla was present wherever hydroxapatite was deposited. Examples include atherosclerotic plaque, tumor calcinosis, subcutaneous calcifications, bursae in the shoulder and calcium-containing renal calculi. The Gla content varied in different sites. An abundant source was atherosclerotic plaque which contained a Gla protein with a molecular weight of 80,000 daltons, whereas in bone very low levels of a Gla protein of 6,000 daltons were found. In kidney stones the scientists found a I7,000 dalton Gla-containing molecule which accounted for nearly 40% of the organic matrix of the renal calculi.

In still other studies designed to determine if urinary Gla content was related to disease, it was discovered that patients with scleroderma and dermatomyositis excreted a 2 to 4 fold higher level of Gla than age- and sex-matched normal controls. Gla was also elevated 20-30% in the urine of osteoporosis patients, and patients with active paraosteoarthropathy showed increased levels of Gla during episodes of ectopic bone formation. In the latter, urinary Gla was decreased when ectopic bone formation was controlled by treatment with diphosphonates. The origin of these vitamin K-dependent proteins has not yet been established.

An investigator at the University of California, Los Angeles, has extended his studies of a purified bone morphogenetic protein (BMP). Trephine defects in the skull of the adult rat were used to test the efficacy of the BMP. These defects do not spontaneously heal, but after they had been implanted with bovine BMP, the defects healed both by ingrowth from the bony rim and by proliferation of perivascular mesenchymal-type cells from the dura mater. Between 3 and 4 weeks after implantation, sinusoids formed and woven bone was remodelled into normal lamellar bone.

At the Medical College of Georgia, investigators have studied the uptake of fluoride isotopes by the developing enamel of the rat incisor. In their *in vivo* studies ^{18}F and ^{19}F were given intraperitoneally in graded doses from 0 to 13mgs F per kilogram body weight. The enamel was divided into developing, transitional, and maturing portions and each portion analyzed for fluoride and phosphorus. Both fluoride isotopes gave the same information. When fluoride uptake was expressed in terms of dry enamel weight, the youngest (developing) enamel accumulated the greatest amount of fluoride and the maturing portion the least. However, when the data were expressed in terms of water content, the developing and maturing enamel were statistically identical in fluoride uptake. In a companion *in vitro* study, with the ameloblasts removed, fluoride uptake by the different enamel portions had the same ratios (developing/maturing) as in the *in vivo* studies. These data suggest that fluoride uptake by enamel occurs at all stages of development by passive diffusion and that ameloblasts may not selectively influence fluorosis or fluoride cariostasis.

NUTRITION

Zinc deficiency in young children is now recognized in many countries including the U.S.A. As a result, the importance of the previously discovered effects of zinc deficiency on skeletal and dental tissue has become increasingly recognized. In recent studies at the University of Alabama, investigators have shown that rats deprived of adequate dietary zinc exhibit reduced glycosaminoglycan metabolism of membranous bone, but no interference with calcium and phosphorus deposition. They also showed in rats that mothers subjected to zinc deficiency continuously during the last week of gestation and the following 18 days of lactation produced pups which developed more dental caries than controls.

In related work at the University of Texas, San Antonio, rats which were subjected to protein calorie malnutrition utilized the available zinc in their diet poorly and became deficient. However, when picolinic acid supplements, at 0.2 gm/Kg diet, were given to the rats on low protein diets, zinc levels in plasma, hair and liver samples were not significantly different from animals on normal diets. The picolinic acid appears to aid transport of the zinc through the intestinal wall of these malnourished rats. This finding will enable researchers to discriminate between cellular changes induced by low zinc and those induced by low protein in oral tissues.

Summary of Research Highlights

SALIVARY GLANDS

Acidic proline-rich protein (PRP) levels in 1 to 24 hour pellicle reflect low rates of plaque formation. An indirect immuno-ferritin technique for detection of (PRP) in parotid and submandibular glands has been developed.

New evidence indicates that fluoride ingested after tooth eruption is cariostatic, and that the source of the fluoride is either the gingival crevice fluid or saliva.

SOFT TISSUES

An *in vitro* method has been developed to measure the permeability of keratinized oral epithelium. This system will be used to test a wide range of chemical substances found in food, beverages, medicines and bacterial toxins.

Investigations indicate that adrenergic agents and cAMP reduce epidermal metabolism and proliferation, whereas increased proliferation was associated with loss of beta adrenergic responsiveness.

Keratinocytes from an oral squamous cell carcinoma can support a lytic infection by adenovirus type 2. This finding indicates that these malignant cells have differentiated only to the level of suprabasal cells. Chemical studies indicate that the regulation of keratin production is altered in these cells.

MINERALIZATION

The rat mandibular alveolar ridge was used as a model to identify the three stages of bone turnover — resorption, reversal and formation — which occur over a ten-day period. First mononuclear phagocyte-like cells accumulate and develop the typical ruffled border. New mononuclear cells then replace the osteoclasts and deposit a collagen-free layer which calcifies to form a cement line. Osteoblast-like cells then deposit collagen-containing osteoid which mineralizes to form new bone.

In studies of bone fractures the protein containing gamma-carboxyglutamic acid (Gla) had a constant Gla to calcium ratio during bone formation. In low density rachitic bone, however, Gla was elevated 20-fold over control bone, whereas the dense fraction of rachitic

bone was essentially the same as control bone. In related studies the Gla-containing protein was present wherever hydroxapatite was deposited, but the Gla content and molecular weight varied from one site to another. Other studies indicate that the urinary Gla content is elevated in osteoporosis patients, scleroderma and in active paraosteoarthropathy.

Bone morphogenic protein (BMP) has been used in rat skulls to produce normal bone in trephine defects which do not heal spontaneously.

Isotope studies indicated that fluoride uptake by enamel occurs at all stages of development by passive diffusion only and thus ameloblasts may not selectively influence fluorosis or fluoride cariostasis.

NUTRITION

Rats born to mothers subjected to zinc deficiency during the last week of gestation and the following 18 days of lactation are more susceptible to dental caries than controls. Studies of zinc utilization showed that protein calory malnutrition interfered with zinc transport through the intestinal wall.

PAIN CONTROL AND BEHAVIORAL STUDIES

Introduction

The mission of the Pain Control and Behavioral Studies Program Branch is to increase our knowledge of dental and orofacial pain and of the behavioral factors involved in dental health and disease. In addition, the program supports research focused on oral-facial motor function and dysfunction and on such oral sensory phenomena as taste and smell.

In its simplest form, pain is a warning signal to the organism alerting it to the existence of a functional or organic problem. Once it has served this purpose, however, pain becomes a major problem in itself, sometimes with severe psychosocial and economic consequences. Although man has always attempted to understand pain and to ameliorate its ravages, we are still far from succeeding in either. In recent years, however, it has become clear that human pain is not just a simple stimulus-response phenomenon. Rather, it is a highly complex experiential combination of sensory-perceptual, emotional and cognitive components with the overall effect resulting from the interaction of individual, cultural and societal factors. Since the human pain response is such a complex multifactorial experience, pain research in humans is becoming increasingly recognized as an endeavor which requires multidisciplinary and interdisciplinary approaches.

Whether an individual maintains his natural dentition for life or suffers from oral diseases which impair function, general health, appearance, and physical comfort depends in large part on personal behaviors. Adopting and maintaining personal oral hygiene, selecting a less cariogenic diet, seeking timely and effective dental care, adopting and adhering to appropriate preventive and therapeutic regimens are behaviors which enable an individual to prevent oral diseases and maintain oral health. Similarly, behaviors occurring within the dental practice setting itself, behaviors occurring within social institutions, such as schools, and decisions reached within communities profoundly influence the public's oral health status.

This program supports behavioral and social science research directly related to improving oral health and increasing the effectiveness and acceptability of dental care. Areas of interest include preventive attitudes and behaviors, factors determining the avoidance or seeking of dental care, behavioral management of fearful or non-compliant dental patients, and psychophysiological studies related to oral conditions and dental treatment. Also of interest are measures of the psychosocial impacts of dental diseases or dental therapies, studies of the diffusion and adoption of new preventive and therapeutic measures, and epidemiological studies. In responding to the challenge to generate new knowledge about the major problem areas identified above, support is provided through a variety of program mechanisms.

Administration

Table 1 shows the distribution of funds for research and research training for FY 1982, by funding mechanism. During FY 1982, the Pain Control and Behavioral Studies Program Branch awarded a total of $3,566,255 for research and research training projects. Of this total $3,157,004 was allocated for research grant support. Awards for the support of research training totaled $398,251. A total of 73 projects were active during FY 1982. Fifty-six projects (43 research grants and 13 research training grants) received funds during FY 1982.

Table II summarizes the distribution of funds within the program by content area. It should be recognized that for some awards considerable overlap exists between categories. In those instances, the primary content area was used for this summary. As indicated in Table II, 21 of the grants awarded during FY 1982 related to basic, clinical, or behavioral aspects of oral-facial pain. Eleven related to orofacial motor and sensory function. Nineteen were for behavioral dental research in non-pain related content areas, such as behavioral aspects of prevention, dental treatment avoidance, and treatment compliance.

TABLE 1 FY 1982 RESEARCH AND TRAINING SUPPORT

BY FUNDING MECHANISM

	No. of Projects		Funds ($000s)	% of Funds
Research Grants	Active	Funded		
Program Projects (P01)	1	0	0	
Regular Research Grants (R01)	39	31	$2,675	75
New Investigator Awards (R23)	8	8	350	9.8
Small Grants (R03)	1	1	15	.4
Career Development Awards (K04)	3	3	117	3.3
Research Conference (R13)	1	0	0	
Totals	53	43	$3.157	88.5
Training Grants:				
Short Term Grants (T35)	5	5	56	1.5
Institutional Grants (T32)	4	3	244	6.8
Fellowships (F32 & 33)	12	5	109	3.1
Totals	21	13	$409	11.4
GRAND TOTALS	74	56	$3,566	99.9%

TABLE II

FY 1982 Distribution of Research and Research Training Funds
by Content Area

Content Area	No. of Projects		Funds ($000s)	% of Funds
	Active	Funded		
I. Pain-related Research				
A. Basic	13	10	$ 555	15.6
B. Clinical	9	6	460	12.9
C. Behavioral	7	5	403	11.3
Totals	29	21	$1,418	39.8
II Oral-Motor/Sensory Research				
A. Oral-Motor	8	7	421	11.8
B. Sensory	5	4	182	5.1
Totals	13	11	$ 603	16.9
III. Behavioral Research				
A. Clinical Syndromes/ Psychophysiology	3	2	49	1.4
B. Behavioral Aspects/ Prevention	5	4	505	14.2
C. Dental Fear and Anxiety	6	4	365	10.3
D. Behavioral Aspects/Dental Treatment	5	5	283	8.0
E. Behavioral Aspects/Disease or Treatment Outcomes	6	4	275	7.7
Totals	25	19	$1,477	41.6
IV. Other	6	5	56	1.6
GRAND TOTALS	74	56	$3,554	99.9%

Staff Activities

During FY 1982, staff engaged in a variety of activities for the purposes of staying abreast of research advances, maintaining close contact with other scientists working in pain and behavioral research areas and continuing their professional development. These activities are listed below:

Participating in Scientific Meetings

Annual Meeting of American Psychological Association, Los Angeles*	Aug 1981
Third World Congress on Pain, Edinburgh	Sep 1981
Annual Meeting of the American School Health Association, Washington, D. C.*	Oct 1981
Council of Graduate Departments in Psychology, Washington, D.C.*	Feb 1982
IADR/AADR Annual Meeting, New Orleans*	Mar 1982
Annual Meeting of the Eastern Psychological Association, Baltimore	Apr 1982
American Dental Association Workshop, Chicago	May 1982
Annual Meeting of the Academy of Behavioral Medicine, Vermont*	Jun 1982

Monitoring, Evaluation, and Site Visits

University of Connecticut, Storrs	Jul 1981
University of Pennsylvania, Philadelphia	Oct 1981
Meeting with State-of-the-Science reviewers in program area, Bethesda	Oct & Nov 1982
University of Washington, Seattle	May 1982

Staff Development

Behavioral Strategies for Supervisors and Managers, Gettysburg	Nov 1981
EEO training, Bethesda	Dec 1981
Effective Supervision Workshop, Bethesda	Mar 1982
White House Workshop, Washington, D.C.	Apr 1982

*Invited Lectures

Research Highlights

TEMPOROMANDIBULAR JOINT DISORDERS

Temporomandibular joint (TMJ) disorders are receiving greater attention from both practitioners and investigators. Epidemiologic studies indicate that TMJ problems affect the general population to a greater degree than previously believed and that the incidence is rising, particularly in younger patients. The most common findings are pain on one side of the face, most often in the TMJ region, limitation or restriction of jaw opening and characteristic "clicking" joint sounds. In recent years NIDR has sharply increased its support of TMJ research, and is currently supporting 8 TMJ research projects totalling $894,000. Two of these projects will be described in detail.

TMJ research at the University of Illinois has demonstrated that most patients with TMJ problems do not have a structural problem with the joint itself, but instead, are suffering from a painful dysfunction of the associated masticatory musculature. Since this condition is now believed to be of complex psychophysiologic origin, treatment should include behavioral as well as other approaches and should avoid or greatly minimize irreversible alterations of TMJ structural relationships.

Despite progress in understanding the basis of a major segment of TMJ disorders, considerable controversy exists over the diagnosis and treatment of these conditions. Many practitioners still believe that all or most TMJ problems are due to mechanical or occlusal problems, rather than psychophysiologic causes; thus many individuals continue to be treated by extensive and irreversible mechanical and surgical methods that often result in long-lasting problems. According to many investigators, these methods represent overtreatment, even when they appear to be successful.

In one NIDR-supported project at the University of Illinois, a group of investigators have attempted to demonstrate the commonalities of the "muscle-pain" TMJ problem, called MPD syndrome (myofascial pain dysfunction syndrome) with other types of psychophysiologic disorders. One of their objectives was to influence practioners to consider behavioral approaches in treating these problems. Treatment included biofeedback, relaxation, and short-term psychotherapy. Some specific findings from this group are given in the following paragraphs.

Before treatment, MPD patients were more sensitive to experimental pain than normal subjects. However, following successful treatment, MPD patients demonstrated a significant reduction in their sensitivity to experimental pain, whereas those patients in which treatment was not successful did not show this change. These findings suggest that relief from pain symptoms is accompanied by a change in experimental pain response toward the normal range, and that differences in pain responsiveness between normal and MPD patients are not due to inherent psychological or physiological differences, but instead, are due to the psychophysiologic effects of chronic pain.

Another study by the Illinois group focused on the families of MPD patients. It has been reported that patients with psychophysiological disorders such as anorexia nervosa, asthma, and certain types of abdominal pain often come from families which are over-protective, over-involved in each other's lives, over-ambitious and overly concerned with success and prestige. A comparison of MPD families with non-MPD families using the Family Concept Inventory Questionnaire indicated that the family characteristics of MPD syndrome patients are very similar to those of the patients with other psychophysiological disorders. These findings emphasize the importance of considering psychosocial factors in the assessment and treatment of MPD syndrome.

Investigators at the State University of New York at Buffalo have been studying the long-term effectiveness of the behavioral technique of EMG biofeedback with chronic TMJ pain patients. Patients selected for this study had had the TMJ disorder for at least 2 years, and had undergone prior treatment which was unsuccessful. Biofeedback treatment was provided for one year and then discontinued. After the one year of treatment, an overall 80% improvement rate had been achieved, and about half of the improved patients were symptom-free. A four year follow-up has now been completed, and the findings indicate that the improvements achieved during the first year have been maintained without further biofeedback therapy.

PSYCHOPHYSIOLOGY OF ORAL PAIN

Investigators at Northwestern University in Chicago have discovered that rats can learn to change their brain waves in areas of the brain known to be related to facial pain. Even more important, impressive reductions in the rats' responses to painful facial stimulation were observed after the rats had been conditioned to alter their brain wave activity.

Small resistors were attached to the rats' faces and varying, carefully controlled levels of heat were applied. Changes in response to the painful heat were measured by recording electronically the number of seconds elapsing before the animal touched its face. Other studies have demonstrated that this procedure

provides an extremely sensitive and reliable index of pain.

The rats learned to alter their brain waves (cortical-evoked potentials) on cue through a carefully sequenced series of conditioning trials on which they received rewards for small changes in brain wave patterns. Only those animals who demonstrated successful conditioning of the cortical-evoked potentials showed consistent reductions in pain responses. With these experimental animals the conditioning procedures produced a dramatic decline in pain responsivity.

In related experiments with human subjects, the same investigators found that patients with a diagnosed chronic orofacial pain condition (myofascial pain dysfunction syndrome) show smaller, more irregular cortical-evoked potentials than observed in normal controls. Investigators are now beginning studies with human experimental subjects to determine if the conditioning procedures developed in earlier research with animals can influence human pain perceptions and responses.

The findings already available suggest that there may be important and readily measureable cortical-evoked correlates of clinical orofacial pain. In other words, we may be close to discovering methods to determine whether and how much orofacial pain a patient has by studying characteristics of his brain waves.

If confirmed and expanded in further studies, such findings could lead toward much needed improvements in diagnostic procedures for chronic orofacial pain disorders. Moreover the initial findings relating cortical-evoked potentials in animals to their pain reactions raise the exciting possibility that we may discover how to control human pain responses through safe, non-pharmacological interventions involving the learned, voluntary control of easily-recorded brain wave activity.

ORAL-FACIAL MOTOR FUNCTION AND DYSFUNCTION

Although clinical evidence indicates that orofacial motor dysfunctions are found in a significant percentage of the U.S. population, no valid epidemiologic studies have been done to determine accurately the prevalence, societal impact and clinical significance of many of these disorders. Since no reliable, standardized diagnostic criteria have been developed, and since much of the symptomatology (pain and limitation of movement) is not perceived to be causally related to an underlying motor dysfunction, these disorders are often ignored or misdiagnosed. As a result, treatment is often empirical and inadequate. When they seek treatment, patients with these disorders may find themselves in a medical no man's land, where they are at the mercy of widely differing, and perhaps diametrically opposed views on diagnosis and treatment. The chief causes of this state of diagnostic and therapeutic confusion can be attributed to the complexities of the clinical problems and to the lack of basic knowledge about the neuromuscular functioning of the oral-facial complex. Despite the essential role of the oral-facial motor system in such basic functions as chewing, biting, swallowing, and speaking, far more is known about the neuromuscular functioning of the knee joint than is known about the oral-facial region.

Although much of the basic knowledge of oral-facial musclature and its control by the central nervous system cannot as yet be clinically applied, significant progress in our understanding of this area has been made in recent years. For example, research at the University of California at Los Angeles has led to the first recording of intracellular activity in jaw muscle motoneurons. This achievement made it possible for scientists to develop a detailed description of the reflex inputs to these motoneurons. It is now possible to describe the organization and latency of such reflexes, and to localize some of the interneurons involved in polysynaptic reflexes. More recently, it has been demonstrated that recordings of intracellular activity in these motoneurons can be obtained in animals that are spontaneously producing rhythmic jaw movements. In the near future such studies may be able to provide preliminary models for the proposed brainstem pattern generator for mastication.

Jaw muscle spindle afferent nerves have long been regarded as important elements in the motor control of the mandible. Two types of nerve fibers, known as primary and secondary, arising from these spindles can be distinguished on the basis of their responses to muscle stretch in anesthetized animals. Primary fibers are generally far more sensitive to stretch stimuli than secondary fibers. The signals (responses) coming from these muscle spindle nerve fibers in normally behaving animals are, however, not well understood. Studies in unanesthetized monkeys supported by NIDR at the University of Washington, Seattle, have made contributions to our understanding of how jaw muscle spindle fibers function. In these animals, primary muscle spindle afferents exhibit exquisite sensitivity to all jaw opening movements. They become silent, however, during jaw closing movements, even those of low velocity. Furthermore, these fibers do not exhibit consistent position-sensitivity. Secondary jaw muscle spindle afferents, on the other hand, fire at a rate which is linearly proportional to muscle length and function in this way in both jaw opening and closing movements. They also fire under conditions of low velocity jaw movement.

The overall result of these differentiated responses by jaw muscle spindle afferents is to provide essential proprioceptive information to the brain so that it can control both jaw position and movement. Current work is focused on the routes by which these signals reach higher brain centers that are concerned with complex oral-facial motor behavior.

The emergence of a concept of central "programming" of complex movement, together with a reassessment of the role of reflexes in producing ongoing movement, has led in recent years to a more sophisticated view of the role of sensory feedback than was held previously. In the past, scientists believed that reflexes were all-important. Now we know that reflexes can be modulated either by control of interneurons or by presynaptic effects, or both. We also know that afferents from muscles and teeth can and do affect motor control during natural movement. It seems likely that as the concept of "pattern generator" takes more definite form, it will include some capabilities for reflex control as well as some elements that allow for long-term modification by proprioceptive sensory information from peripheral receptors.

DENTAL FEAR AND ANXIETY

The information emerging from research currently supported by NIDR has important implications for the prevention, assessment, and treatment of dental anxiety. Anxieties concerning dental treatment are very widespread; not only do they cause considerable personal anguish, but they also make the dentist's tasks of providing preventive and restorative care and effective pain control more difficult. For some patients — an estimated 10-15% of the adult public — dental anxiety is sufficiently intense to cause significant treatment delays or complete avoidance of dental care.

Researchers at Kent State University have studied the prevalence of dental anxiety in a large sample of college students who completed four different dental anxiety questionnaires. Between 70 and 80% of the college students reported at least some anxiety, and 10-20% reported very high levels of anxiety about dental treatment. Investigators concluded that wider utilization of available dental anxiety screening measures could improve the management of fearful or treatment-avoiding patients and ultimately improve the public's utilization of dental services.

Since dental anxieties often have their inception in childhood, it is important to study childrens' responses to treatment. Recent research conducted at the University of Florida indicates that showing a carefully prepared videotape to children can reduce dental anxiety. The tape provided first-time dental patients, 5 to 12 years old, with detailed instructions on how to practice stress management techniques such as controlled breathing, and how to use visual imagery distraction. These children showed significantly lower levels of physiological arousal, self-reported anxiety, and behavioral disruptiveness during subsequent dental treatment than did children receiving routine preparation.

Although some strategies for managing fearful and uncooperative children have been widely taught in dental schools and used in dental practice, the efficacy of these approaches has not been adequately tested. Therefore, investigators at the University of West Virginia are also studying how dentists can reduce disruptive behaviors in the most efficient manner. In these studies, the investigators are trying to develop simple procedures which require little professional time or training and can be used conveniently in routine practice.

Since early childhood experiences with dentistry often color later attitudes about oral health and dental treatment, the investigators have studied patients between the ages of 3 1/2 and 9 years, who were receiving dental care in a dental school clinic. Each child required at least two visits for restorative work. During each visit a trained observer described the various disruptive and cooperative behaviors the child exhibited and recorded the duration of each behavior. The behavior coding system used had previously been tested and met high standards of reliability and validity.

During the initial restorative visit the dentist provided treatment as it was usually provided in the clinic. On the second visit, the children were randomly assigned to one of three treatment conditions. Children assigned to the "Control" condition again received routine restorative treatment. Children assigned to a "Continuous Audiotape (Distraction)" condition were supplied with earphones, through which they heard childrens' stories read continuously throughout the dental visit. For children in a third "Contingent Audiotape" condition, the tapes were played only when the child was quiet and cooperative. The dentist controlled the audiotapes with a foot switch and reported that this activity was easy to combine with the usual dental treatment.

Children treated under the "Contingent Audiotape" condition showed a large reduction in disruptive behaviors. When the duration of disruptive behaviors during visits I and II were compared for each child, these children showed an overall 70% reduction in disruptive behavior time. In contrast, children hearing the tapes continuously showed only a modest reduction (33%). Control subjects showed no differences between disruptive behavior time from the first visit to

the second. Moreover, the children who could hear the stories only while they were showing cooperative behaviors (Contingent Audiotapes) actually showed less anxiety during and after treatment, as measured by standardized methods for assessing dental anxiety in children.

Although the efficacy and practicality of this intervention have not yet been tested in a typical private practice setting, the results to date clearly demonstrate that as simple and inexpensive intervention can significantly reduce disruptive behaviors and anxiety in children receiving restorative dental treatment.

PSYCHOSOCIAL CORRELATES OF ORTHOGNATHIC SURGERY

A project at the University of Washington is studying outpatients in an Orthognathic Surgery Clinic. It has two objectives: 1) to learn how patients who decide to undergo surgery differ from those who choose orthodontic treatment only, and how they differ from those who decline further treatment altogether, and 2) to find out how pre-surgical expectations are related to post-surgical satisfaction or dissatisfaction with the treatment outcomes.

The results indicate that patients who decide to undergo surgery see themselves as having significantly greater problems with oral function, occlusion and appearance than those in the other two groups; the surgical patients also scored significantly lower on a self-rating of facial image. Pre-surgical patients as a group tend to expect improvements across many areas of their lives, including oral function, general health, appearance and interpersonal relationships. These patients will be followed longitudinally to determine how their initial expectations and later satisfaction with orthognathic/orthodontic treatment are related.

PREVENTIVE BEHAVIOR AND ATTITUDES

Studies supported by NIDR are making progress in identifying behavioral factors which are important in the prevention of oral diseases, especially caries and periodontal diseases. Current efforts to prevent these widespread diseases depend in large part on patient behaviors, such as using a fluoridated dentifrice, rinsing with a fluoride solution, drinking fluoridated community water, selecting a less cariogenic diet, and practicing adequate oral health hygiene; these efforts also depend on practitioner behaviors, such as using effective preventive measures and advising patients to use them.

Our knowledge of how to produce and sustain preventive behaviors is still in its infancy, but useful leads are emerging from current research. For example, researchers at the University of Connecticut are studying factors that determine whether or not adolescents use a fluoride mouth rinse at home. Earlier retrospective studies with adults had demonstrated that preventive health actions taken by an individual could be correlated with personal beliefs about health. For example, if an adult considered a disease important and felt vulnerable to it personally, the individual would be more likely to take preventive action. However, these new prospective studies in adolescents show that measures of health beliefs do not correlate with follow-through behavior on the home fluoride mouth rinse regimen. Thus, in adolescents, oral health beliefs and oral behaviors do not appear to be correlated.

These investigators also found that motivational strategies which include small tangible rewards usually resulted in acceptable levels of fluoride mouth rinsing over a 20-week interval. Additional behavioral/ motivational strategies currently being evaluated hold promise for producing high levels of sustained compliance over longer periods.

The behaviors and attitudes of 7-and 8-year-old children participating in a fluoride mouth rinse program, as well as the health attitudes and behaviors of their mothers, are also being studied. The results show that simple recording and self-reinforcement procedures, which can be easily taught to young children, greatly increase consistency in use of the fluoride mouth rinse.

Other prevention-related research supported by the program is determining how knowledge on the prevention of bacterial endocarditis through antibiotic prophylaxis is diffused among, and implemented by dental practitioners. In this research, investigators at the Albert Einstein College of Medicine in New York are studying responses from a large sample of general dentists and oral surgeons to determine whether they prescribe antibiotics to prevent endocarditis, and what factors are involved in their decisions. The characteristics of early and late adopters as well as the organizational characteristics of the setting in which they practice will be identified.

Future Plans

During FY 1983 key research areas encompassed within this program's mission will continue to receive support. Multidisciplinary and interdisciplinary approaches to pain research will continue to be emphasized as a means of linking basic and clinical pain studies. In clinical pain studies, the behavioral aspects of the human pain response will continue to receive attention, particularly in areas of pain

assessment and therapy. A special effort will be undertaken to remedy the existing unsatisfactory state of our knowledge of TMJ disorders, particularly in the areas of diagnosis and treatment. Since TMJ disorders are of increasing concern to the American public, NIDR will continue to exercise a key leadership role in identifying the most promising areas for additional research.

Basic neurophysiologic and neurochemical studies of the trigeminal system responsible for innervation of the orofacial region continue to be fruitful and deserve continued support. Attention will also be given to stimulating research into the clinical pharmacology of intravenous sedation for dental purposes. These efforts will be aided by the publication of the proceedings of an NIDR-supported conference-workshop convened at the 1983 annual meeting of the American Association for Dental Research.

Behavioral research currently supported by the program has attained a high level of scientific quality. During FY 1983 this research will be expanded to include previously neglected subjects, such as the process by which new preventive measures are diffused and adopted. The two institutional training grants in behavioral science awarded during FY 1982 will be monitored closely to ensure that trainee and faculty efforts address significant issues in dentistry and the programs produce investigators with outstanding research skills. These training programs are particularly important because progress in this area has been slowed by the paucity of such investigators in the dental field.

Additionally, programming efforts will be made to encourage senior fellowship (sabbatical) applications from experienced biomedical and behavioral scientists. Announcements highlighting opportunities within the senior fellowship program will be prepared for major professional journals and information on this and other research support mechanisms will be disseminated at major scientific meetings.

A program announcement outlining scientific areas in which research support is available will be issued during FY 1983. In addition, a request for grant applications (RFA) focusing on patient adherence to preventive and therapeutic regimens is currently being planned.

During FY 1983 major state-of-the-science reviews prepared as part of the program's research planning efforts will be published and distributed by an international dental organization. These measures should further assist in disseminating timely information about specific research opportunities and needs to the national and international scientific communities.

Summary of Research Highlights

TEMPOROMANDIBULAR JOINT (TMJ) DISORDERS

TMJ disorders are receiving increasing attention. It was recently shown that most TMJ patients do not have a structural problem with the joint, but suffer from a masticatory muscle dysfunction believed to be psychophysiologic in origin. Studies supporting this concept have shown that patients with the TMJ disorder myofascial pain dysfunction syndrome (MPD) come from families which are over-protective and over-involved in each other's lives. When biofeedback treatment was given for one year to chronic TMJ patients and discontinued, 80% improved and 50% of the improved patients were symptom-free. A four-year follow up showed that these results had been maintained.

PSYCHOPHYSIOLOGY OF OROFACIAL PAIN

Recent studies indicate that rats can learn to change their brain waves in areas of the brain related to facial pain. In subsequent experiments, using conditioning procedures, rats showed impressive reductions in responsivity to aversive facial stimulation. Studies are now under way to determine if similar conditioning procedures can influence human pain perception and response. These studies suggest that there are measurable cortical-evoked correlates of orofacial pain and may lead to improved diagnostic procedures and to methods of controlling the human pain response.

OROFACIAL MOTOR FUNCTION AND DYSFUNCTION

Since clinical evidence indicates that the prevalence of orofacial motor dysfunctions is significant, the diagnosis and treatment of these disorders must be improved by research on the underlying neuromuscular defects. Research in animals recently achieved the first recording of intracellular activity in jaw muscle motoneurons, a finding which made it possible to develop a detailed description of the reflex inputs to these neurons. Other studies are focusing on the CNS pattern generator for the jaw muscles involved in mastication. In studies to understand how jaw muscle spindle fibers function, two types of such fibers have been identified. Apparently, these fibers provide proprioceptive information to the brain so that it can control both jaw position and movement.

DENTAL FEAR AND ANXIETY

The prevalence of dental anxiety was determined in college students who completed four different

questionnaires. Between 70 and 80% of the students reported some anxiety about dental treatment, and 10-20% reported high levels. Investigators concluded that wider utilization of anxiety screening measures could improve the management of fearful or treatment-avoiding patients.

Since dental anxiety often starts in childhood, investigators are focusing on childrens' responses to dental treatment. In one project, the investigators prepared first-time dental patients, 5 to 12 years old, by using a videotape which shows a child practicing stress-reducing techniques. In subsequent appointments, these children showed less anxiety.

Another study showed that disruptive behaviors in young patients can be reduced by having the child listen to story-tapes during treatment. Playing the tapes continuously (distraction) produced some improvement. However, if the tapes were played only when the child was quiet and cooperative (contingent condition), both disruptive behaviors and anxiety were greatly reduced.

PSYCHOSOCIAL CORRELATES OF ORTHOGNATHIC SURGERY

Investigators are studying the differences between patients who accept orthognathic surgery and comparable patients who do not. Surgical patients believed that they faced worse orofacial problems than the controls, and also believed that their lives would improve in many areas following the surgery. These patients are being followed to determine the relationships between their initial expectations and their subsequent attitudes about orthognathic/orthodontic treatment.

PREVENTIVE BEHAVIORS AND ATTITUDES

Motivational programs involving the use of rewards caused improvements in both short-term and long-term adherence to a fluoride mouth rinse regimen. These studies also showed that, in adolescents, health belief and attitude measures do not predict which subjects will follow through in using a fluoride mouth rinse.

Another study is determining whether dental practitioners prescribe antibiotics to prevent bacterial endocarditis after oral surgery, and what factors are involved in their decisions. The objectives are to improve dentist adoption of preventive practices related to bacterial endocarditis, and to find out how new information on prevention is diffused and adopted.

Part D

NATIONAL INSTITUTE OF DENTAL RESEARCH ANNUAL REPORT

Intramural Research Program

October 1, 1981 - September 30, 1982

INTRAMURAL RESEARCH

NATIONAL INSTITUTE OF DENTAL RESEARCH

October 1, 1981 - September 30, 1982

REPORT OF THE DIRECTOR

It is the continuing responsibility of the Intramural Research Program to initiate and conduct basic and clinical research programs in areas of importance to dental health, and to train researchers who can extend these studies both inside and outside the NIH to form the base for major advances in dental and biomedical research.

These efforts in the past have led to a definition of the potential role of immmunological mechanisms in periodontal and other chronic inflammatory diseases; the identification and biochemical characterization of tissue specific collagens, proteoglycans, and attachment proteins; the etiology and transmissibility of dental caries; and the development of new techniques allowing early x-ray detection of small changes in tooth and bone mineral, to name a few. Ongoing research pursues in depth many of these early discoveries as well as studies on acute and chronic pain; diseases of the oral soft tissues; salivary gland structure and function; mineralized tissues in health and disease; ecology, metabolism and physiology of oral microorganisms; and the role of environmental agents and genetic factors in oral-facial malformations.

To conduct this research the Intramural Research Program is divided into eight laboratories and branches and the reader is referred to the summary reports for a more detailed description of the ongoing research. This past year's research has led to many significant advances, but has also seen the departure of several key staff members necessitating a reevaluation of organizational structure. The results of these deliberations and other administrative actions of consequence to the Intramural Research Program are the main topic of the remainder of this report.

During 1981-82 three distinguished members of the senior staff who together had served the Institute over 75 years retired. In February, Dr. Karl A. Piez, who had been Chief of the Laboratory of Biochemistry for 15 years, left the Institute after more than 30 years of continuous service to become Director, Research and Development, Collagen Corp., Palo Alto, California. Dr. Piez's outstanding research on the biochemistry and structure of collagen brought national and international recognition to the NIDR and the NIH and set a benchmark of excellence for all the other research programs of the Institute's Intramural Research Programs.

In May, Dr. Paul H. Keyes, Dental Director, retired from active service after more than 25 years with the NIDR to join the International Dental Health Foundation, Reston, Virginia. Dr. Keyes' research on the etiology and transmissibility of dental caries, conducted during the 1960s, stands as one of the milestones in dental research. His more recent studies on periodontal disease aimed at developing and testing new diagnostic and treatment modalities have once again revealed his fundamental understanding of the processes involved in the etiology and pathology of the two most common chronic diseases of mankind, caries and periodontal disease. This work has already spurred many scientists and clinicians to reexamine ideas about and approaches to the treatment of periodontal disease.

Finally, at the end of the fiscal year, Dr. James F. Bosma, Chief of the Oral Pharyngeal Development Section, Diagnostic Systems Branch, retired after 21 years of service with the NIDR to become a consultant to the Swallowing Center at the Johns Hopkins University School of Medicine, Baltimore, Maryland. Dr. Bosma, during his tenure at the Institute, made many significant contributions to our knowledge and understanding of the anatomy and function of the oropharyngeal complex, making it feasible to diagnose and treat dysfunctions affecting this part of the body. His research has, in fact, provided much of the scientific underpinnings for the Center he now joins.

Mr. Terry Medlin, Chief, Scientific Systems Section, left the Institute after 10 years to accept a position at Gejac Co., Riverdale, Md. He was instrumental in developing the current data processing facilities and services for the research and clinical programs of the Institute. Shortly before Mr. Medlin left, the Section was transferred to the Office of the Director for Intramural Research, a logical and beneficial move since its major function is to serve the Intramural Program. In August of this year, Ms. Sheila Taylor was appointed Chief of the Scientific Systems Section.

The research program in taste, initiated under Dr. Bosma's sponsorship, was transferred earlier in the year to the Clinical Investigations and Patient Care Branch. Therefore, with Dr. Bosma's retirement, the Section of Oral Pharyngeal Development becomes

inactive and will be dissolved. Since this move makes it unnecessary to maintain the Branch's other section, the Diagnostic Methodology Section, that too will be abolished. The research of the latter Section, however, will continue. In fact, plans are under way to expand the program into clinical studies involving the application of new and improved diagnostic methods in dentistry.

The departure of Dr. Piez presented the Institute with somewhat different choices. The Laboratory of Biochemistry had developed a three-section structure with Dr. Piez himself heading the Protein Chemistry Section. It was apparent that as the Section programs had developed and matured, they had grown apart to some degree, sharing some goals and facilities with programs of other laboratories in the Institute. Out of these collaborative efforts had come significant new findings warranting further exploration and support. It was against this background that an Advisory Committee was set up composed of Dr. Abner L. Notkins, Chief, Laboratory of Oral Medicine, Dr. George R. Martin, Chief, Laboratory of Developmental Biology and Anomalies, and Dr. Arthur R. Hand, Laboratory of Biological Structure, to advise the Scientific Director on the future of the Laboratory of Biochemistry. The Committee reviewed the history of the Laboratory and its current research as well as carried out interviews with members of the Laboratory, other members of the Institute, and outside consultants. After considering a number of options, a set of recommendations for reorganization was presented to and accepted by the Scientific Director who in turn received tentative approval from the Acting Director, NIDR, the Acting Deputy Director for Science, NIH, and the Director, NIH, to go ahead with the proposed reorganization. The proposal was also reviewed and endorsed by the Board of Scientific Counselors during their Meeting in April.

As a direct outcome of these events, it is anticipated that the following changes will become effective at the beginning of the new fiscal year: Abolishment of the Laboratories of Biochemistry and Biological Structure and in their stead the creation of two new programs, a Laboratory of Oral Biology and Physiology (LOBP) and a Mineralized Tissue Research Branch (MTRB); appointment of Dr. Hand as Chief, LOBP; and initiation of a nationwide search for a scientist to head up the MTRB. Of the seven Sections which make up the two existing laboratories, the Protein Chemistry Section will be abolished while the other six sections are to be divided between the two new programs as indicated by program relevance.

In their proposal the Advisory Committee placed special emphasis on the creation of the Mineralized Tissue Research Branch, listing the following specific reasons. A. Bone and hard tissue are critically involved in development of oral tissues and represent major sites of oral diseases. B. Existing NIDR units are currently at the forefront of research in this and closely related connective tissue fields. C. Unification of existing units working in relevant areas would create the most significant multidisciplinary effort in bone and hard tissue research in existence at NIH. D. Unification and coordination of these efforts should accelerate research progress in this area and serve as a visible center for NIDR efforts without requiring major new commitments in personnel and space. Similar opinions were expressed by the Board of Scientific Counselors who also stressed that the reorganization would bring together in the new laboratory of Oral Biology and Physiology activities important to the Institute.

Substantial progress continued to be made during the year toward building a strong clinical dental research program. The first move toward the achievement of this goal was made in FY 79 with the appointment of Dr. Karl-Ake Omnell as Clinical Director and the subsequent establishment of a Clinical Investigations and Patient Care Branch (CIPCB) with Dr. Omnell as its chief. Dr. Omnell resigned in June 1981 and as one of his last acts recommended that the responsibility for Patient Care and Clinical Investigations be divided between two individuals. This led to the establishment within the Branch of two Sections, a Patient Care Section and a Clinical Investigations Section, and the appointment of Dr. Michael W. Roberts as Chief, Patient Care Section in August, 1981. During the year since his appointment, Dr. Roberts has continued the efforts initiated by Dr. Omnell to upgrade both the management of the Dental Clinic and the services it provides to the other Institutes and to the NIDR. Notable among achievements this year is the development and implementation of a data collection system that will permit compilation of epidemiological and patient services data for potential research and patient management use.

On January 1, 1982, Dr. Bruce Baum, previously of the National Institute on Aging, took over as Clinical Director and Chief, CIPCB and Clinical Investigations Sections. A Section staff was assembled both from persons new to the Institute as well as by transfer of individuals to the Branch from other laboratories and branches within the Institute. July 1, 1982, the Section occupied newly renovated laboratory space in the Clinical Center.

Development of plans not only for the renovation of the laboratory space but also for a subsequent renovation of the Dental Clinic itself has consumed much time and energy during the year. In addition, considerable efforts have been expended on developing new guidelines for

the Dental Staff Fellow Program (formerly the Associate Training Program) and distributing initial notices about this program to the Nation's Dental Schools and extramural Clinical Training Programs. The Dental Staff Fellow Program is an essential ingredient in the Institute's plan to build a strong clinical program. At the same time, it offers unique training opportunities to young dentists interested in a career in dental research. The fact that so many qualified candidates applied this first year, in spite of the newness of the program, bodes well for its future.

Significant progress was made this year on pain mechanisms and pain control in the Neurobiology and Anesthesiology Branch, the one intramural program that already has a strong clinical component. In addition to exciting new findings on placebo effects and the role of stress in producing postsurgical analgesia, these clinical investigators have initiated collaborative pain studies with other Institutes, including pain associated with cancer and diabetes. It had become increasingly clear that the Dental Clinic was not a suitable environment for conducting these broadly-based clinical pain studies. The program also was severely restricted by the limited space available in the Clinic. For these reasons, in 1979, the Institute requested that the Clinical Center, NIH, establish a pain research facility in the ACRF with the NIDR as the lead Institute. A plan for such a clinic was submitted and modified in 1980. The request was approved this year and discussions are currently underway regarding space and staffing needs. The present schedule calls for the pain program to move to the ACRF and be in operation this fall.

Other intramural programs have made important basic discoveries that are ready to be explored further in a clinical setting. As an example, data recently obtained establish the existence of a number of non-collagenous bone matrix proteins that in contrast to collagen are unique to mineralized tissues. These findings provide the impetus for studies evaluating the role of these constituents in terms of diagnosis and treatment of human bone disease. Studies on mechanisms of attachment of cells to tooth surfaces have shown that fibroblasts use the attachment protein fibronectin to bind to the collagen of the root while epithelial cells use another attachment protein, laminin to attach to the tooth. These observations have resulted in the development of a treatment concept which will shortly be tested in patients with periodontal disease. The demonstration that the concentration in gingival fluid of a growth factor for lymphocytes produced by oral mucosal epithelial cells correlates with the extent of inflammation in the gingiva may provide a new diagnostic tool. These findings together with other basic research studies may also provide clues leading to an understanding of the etiology and pathogenesis of diseases of the oral mucosa, including cancer. Recommendations regarding how best to pursue these leads are being formulated by a small internal committee, chaired by Dr. Stephan E. Mergenhagen, Chief, Laboratory of Microbiology and Immunology. Their report, due in October 1982, is expected to guide future developments of Intramural Research program efforts in these important areas.

The above review undoubtedly leaves the impression that the Intramural Program of the NIDR is prospering. The Summary Reports of the Laboratories and Branches which follow seem to confirm this point as they describe many new and significant achievements. However, while the programs are as scientifically strong as ever, their appearance of good health hides the grim truth they are running out of funds with which to conduct research. A recent analysis of intramural funding at NIH shows that while in the aggregate intramural research has grown 11.2% in constant dollars in the period 1977 to 1983, the NIDR intramural program suffered a 10.3% decline during the same period. Preliminary analysis of the FY 83 budget makes it quite clear that this downward trend for NIDR is continuing. Unless other means are found to increase funding, it will be necessary to begin cutting back on our efforts. Options that are being or will be pursued are 1) reimbursement of costs associated with delivery of dental care to patients of the other Institutes, 2) outside funding (Foundations, Industry, Universities) of post-doctoral fellows, and 3) expansion of collaborative efforts with laboratories in other Institutes and outside NIH.

As the project reports show, the programs have already been quite successful in securing outside support for research fellows and in promotiong collaborative efforts inside and outside NIH. This success is a measure of the high regard in which they are held as are the numerous invitations extended to staff members to participate, at no cost to the Government, in national and international conferences. A recently published list of the 1000 contemporary scientists most cited 1965-1978 included the names of four NIDR intramural scientists, Dr. George R. Martin, Dr. Stephan E. Mergenhagen, Dr. Joost Oppenheim, and Dr. Karl A. Piez. Dr. Stephan E. Mergenhagen also was the recipient of the Periodontal Disease Research Award of the International Association of Dental Research. Further, Dr. Stephen Gobel was awarded the PHS Commendation Medal, Dr. Abner L. Notkins was elected to membership in the Association of American Physicians, and the American Board of Oral and Maxillofacial Radiology conferred diplomate status on Dr. Richard L. Webber. Mr. Frederick J. Brown and Ms. Marie J. Munsterteiger were recognized for sustained

efforts on behalf of Intramural Program with the NIH Merit Award. The contributions of a number of other employees were recognized through cash awards, EEO Awards, quality increases, elections and/or appointments to professional societies, editorial boards, and advisory committees.

The list of people who have been so recognized reaches across all the programs and include employees of all levels, highlighting the fact that the progress which has been made, is very much the result of a team effort. In reality, all of the programs are understaffed, and it has taken dedication and sustained effort by everyone to maintain the high caliber of research which continues to be the hallmark of the NIDR Intramural Program. It is doubtful, however, that we can carry on at this level much longer. The measures outlined previously may give some relief if successfully accomplished. Nevertheless, the real reason for our present difficulties lies in the steady decline since 1972 of funding for the Institute itself as measured in constant dollars. A reversal of that trend must occur in order to give the Intramural Program the support it merits.

SCIENTIFIC SYSTEMS SECTION

The Scientific Systems Section has just completed its first year as part of the Intramural Research Program at the National Institute of Dental Research. The Section has continued to provide data processing support and consulting services to the NIDR research community by operating the NIDR 11/70 central computing facility, and by providing support for the other dedicated laboratory systems in our Institute. We have attempted to maintain our expected level of support, even though the Section has experienced a significant decrease in staff due to the resignations at the beginning of this fiscal year of both the Section's chief and a part-time programmer in the group. Remaining staff consists of two full-time programmers, one of whom is now the group leader, two part-time programmers, an engineering technician, a computer operator, and a part-time secretary.

Several major projects which will be described below were undertaken and completed this year, as were numerous smaller projects not detailed here. In addition, classes in both the BASIC programming language and the RS1 interactive graphics and statistics package were offered to our user community.

A Workload Reporting System has been developed for the Dental Clinic. A DEC VT100 terminal located in the Dental Clinic in Building 10 has been connected to the NIDR 11/70 computer system using a local area data set connection which provides a 9600 baud (high speed) data transfer rate over a dedicated telephone line. The system was designed to present a comprehensive analysis of Clinic operations by providers and institutes. Interactive data acquisition is accomplished using DEC's FMS - the forms-oriented video input/output management system - and screens similar to the paper fill-in-the-blank forms used in the Dental Clinic. The Workload Reporting System generates the following five reports: Diagnostic Procedures by Institute, Diagnostic Procedures by Provider, Patient Care by Institute, Total Encounters by Provider, and Total Workload Units by Provider. In addition, the data base has been interfaced to Datatrieve, a data base reporting and query system, to accommodate other information needs.

A Matrox Image Display System has been installed on the Neurobiology and Anesthesiology Branch's DEC 11/40 computer system located in Building 10, Room 2B07. Two Sanyo screens have been connected to the system; one is located in the Quiet Room of the Dental Clinic for presenting subject tests, the other is located near the computer and is being used now for program development. An RSX-11M driver which handles both displays has been incorporated into the NAB 11/40's operating system. A comprehensive subroutine library has been developed for displaying points, characters, vectors, and also to implement multiple cursor systems for the Matrox. A major program package has been developed which uses the Matrox System to display three types of Clinical Pain Measurement Scales. A main program invokes any or all of the scaling programs which present tests to the subjects with a minimum of operator intervention. One program records an analog response to extremes of sensation by allowing a subject to manipulate a mercury column in a thermometer. The others allow a subject to manipulate a cursor to select a word in a list, and also to respond by sliding the cursor along a horizontal scale. Cursor movement is controlled by a terminal keyboard.

A Guilford 2400S spectrophotometer in the Laboratory of Microbiology and Immunology, located in Building 30, Room 312 has been connected to the NIDR 11/70 computer system using an Intel microprocessor interface. The microprocessor and a Texas Instruments 743 terminal are hardwired through a common terminal line to the 11/70. A switch box on the microprocessor allows the user to turn off the computer interface, reset the microprocessor, select dwell change or 5, 10, or 20 second continuous sampling, and start and stop data collection. Software has been written for the microprocessor to collect data from the spectrophotometer, format the data, perform error checking, and send the data to the 11/70. Programs running on the 11/70 acquire data from the microprocessor, and perform data analysis.

The Neurobiology and Anesthesiology Branch's neurophysiology laboratory located in Building 30, Room B4, has been interfaced to the NIDR 11/70 computer system. An Intel microprocessor is used to collect neural events into 100 millisecond bins, and to detect the initiation and termination of various stimuli. Stimuli scanned for include switch settings and button presses and releases to enable entry into the system of manual stimuli, and electrical stimuli from stimulus generators which are input automatically into the microprocessor. Data from the microprocessor is transmitted over a terminal line at a speed of 300 baud to the data acquisition software running in the 11/70 where it is stored for later analysis. Graphical analysis software has been written as well.

Work has continued this year on the automation of the Neurobiology and Anesthesiology Branch's B8 neurophysiology laboratory located in Building 30. This ongoing project, which has been continued from the previous year, became fully operable this year. The laboratory is connected to the NAB DEC 11/34 computer using an Intel microprocessor as the interface between the laboratory equipment (i.e.,buttons, lights,

heat probes, and neural and EMG events) and the 11/34. Commands are issued by the software running in the 11/34 to the microprocessor, which performs the actual manipulation of laboratory equipment, time stamps the requested event to an accuracy of one millisecond, and checks for laboratory input, as well as neural and EMG input, all of which are time stamped as well. Data is then returned to the 11/34 for storage. Program flow is determined by parameter files which define the trials to be performed. These, and other run parameters, may be changed after any trial has been executed. Running simultaneously with the experiment control program is on-line graphical analysis software. This software allows the user to view current data graphically in real-time. The current trial may be displayed individually, or categories of trials may be displayed cumulatively. Trial display parameters may be changed in real-time as well. After experiment completion, data is moved to the 11/70 for further analysis, such as behavioral and graphical analysis, and long term storage.

Chromatography analysis software has been written for the Laboratory of Biological Structure to provide scientists with a simple, self-contained, interactive system which could be used by a non-programmer to explore and analyze peak profiles of a variety of chromatography data. Data to be analysed is input to the NIDR 11/70 computer using the Summagraphics digitizer. The program can graphically display the entire chromatographic diagram or a selected region only. Peaks are manually selected by the user; the program then computes the peak area, mean, standard deviation and ratio of the peak area to total area. The user is also permitted to select the display height of a specific peak; the rest of the peaks are normalized accordingly, thereby enabling the user to standardize a number of graphical displays. Data can then be transferred using a telephone link to the DEC 10 so that MLAB may be used to perform a least squares fit on these complex curves with overlapping peaks.

New directions for next year will include offering the user community assistance in the areas of computer generated graphics and posters in an effort to reduce costs incurred in the manual production of such artwork. Another major initiative will be a study of all NIDR computer systems with respect to the replacement of aging hardware and/or upgrade for expanded capabilities.

MICROBIAL SYSTEMATICS SECTION

The Microbial Systematics Section is charged with establishing a data bank for information describing diverse strains of microorganisms. Special emphasis is placed on the human oral microbiota. For this purpose, collaborative projects are on-going with microbiologists distributed throughout the world.

At present there are tens of thousands of scientists, physicians, public health personnel, and others involved in some aspect of microbiology. The number of microbial strains isolated, characterized, and (in many cases) preserved, by individuals runs into the millions. Hundreds of millions of bits of information have been developed on these strains. However, these data are not resident in a single, centrally located system, permitting rapid and efficient utilization. Because of the large volume of information involved and because, in several applications such as classification and identification, mathematical manipulations of the data are required, electronic processing of these data is necessary.

In collaboration with personnel of the American Type Culture Collection, the Food and Drug Administration; the Centers for Disease Control, the Veteran's Administration and numerous academic microbiologists, strain data are being entered into the data bank which provides such services as: data on specific organisms and/or groups of organisms, location of strains with special characteristics, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids for research purposes, aids in quality control of tests, methods, and laboratories, and communication of data via common format.

Data files of primary data on a large number of microorganisms found in the oral cavity and related types are established. These files provide a resource for asking both ecological and epidemiological questions of interest in dental research.

Programs have been developed and tested to enter, retrieve, and analyze the data in a variety of ways for epidemiological, diagnostic, taxonomic, ecological, etc., uses. The long term goal is to establish a world-wide data bank at a series of cooperating centers. As experience grows, better programs are being designed and implemented.

The system originally developed for bacteria is now being expanded to include the yeasts, molds, algae and protozoa. A series of monographs describing the expanded system is in varying stages of publication.

Extensive files of descriptions of filamentous and pleomorphic organisms are being assembled. The files cover all the described types of *Mycobacteria*, blend into the *Nocardia*, then through the *Actinomycetes* (especially a unique set on oral isolates), and finally, *Bacterionema*. An extensive cooperative study has been initiated to study the oral pleomorphic bacteria (many of which are associated with disease). The study will provide a standard set of well characterized bacteria for the Dental Research community. The data from this study will be incorporated into the files on pleomorphic organisms. These files are being actively used in collaboration with the submitters of the data as well as numerical taxonomists to revise the badly confused taxonomic relationships of these bacteria. Such revision is necessary to avoid the misidentification (leading to erroneous epidemiological conclusions) which are found in some recent dental research literature.

Other files on non-filamentous oral organisms (streptococci, lactobacilli, veillonella, etc.) are being constructed to study correlations among caries activity, phenetic span of characters, serology, source of isolation, and host descriptions.

One of the long term goals in establishing all these files is the establishment of probability tables to allow computer-aided probabilistic identification of oral isolates. Probability matrices, for on-line identification of bacteria (including Gram negative rods, lactobacilli, streptococci, bacilli, etc.) have been constructed. They are available to research workers for use.

MICROBIAL SYSTEMATICS SECTION

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Z01 DE00044-12 ODIR

COOPERATING UNITS: R. Gryder, Food and Drug Administration
F. Benedict, EDRO, Food and Drug Administration
R. Gherna, American Type Culture Collection
D. Brenner, Centers for Disease Control
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J. Brooks, Centers for Disease Control
L. Wayne, Veterans Administration
V. Sutter, Veterans Administration
R. Atlas, University of Louisville
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M. Hawman, UCLA
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 D. 00044-12 ODIR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Handling of Microbial Strain Information by Computers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Krichevsky, Micah I.	Research Chemist	ODIR	NIDR
OTHER:	Love, Leslie L.	Information Specialist	ODIR	NIDR

COOPERATING UNITS (if any)

see attachment

LAB/BRANCH

SECTION: Microbial Systematics Section

INSTITUTE AND LOCATION

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Microbial strain data are being entered into a data bank to provide: data on specific organisms, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Data files of primary data on microorganisms found in the oral cavity and related types are established, providing a resource for asking both ecological and epidemiological questions in dental research. Codino conventions have been developed to relate oral clinical parameters with the incidence and distribution patterns of specific microflora. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

Programs are being developed to enter, retrieve, and analyze the data for epidemiological, diagnostic, taxonomic, ecological, etc. uses. The long term goal is to establish a world-wide date bank at a series of cooperating centers. The original bacterial system is being expanded to include the algae, yeasts, molds, protozoa, and hybridomas.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 OE 00ZSO-05 ODIR

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Algorithms for Microbial Systematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Walczak, Cynthia A.	Computer Programmer	ODIR	NIDR
OTHER:	Krichevsky, Micah I.	Research Chemist	ODIR	NIDR
	Mercer, Paula	Computer Programmer	ODIR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION: Microbial Systematics Section

INSTITUTE AND LOCATION: National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MANYEARS: | PROFESSIONAL: | OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Algorithms are being developed and tested for aiding in numerical taxonomy of feature by strain matrices too large to be analyzed by existing programs. Both segmentation and heuristic approaches are being investigated.

A program has been designed to compare and evaluate methods and/or laboratories when characterizing the same set of strains. The usual statistical packages are not useful because of the predominantly binary (i.e., discontinuous) nature of the data. The algorithm allows comparison of tests or laboratories at the levels of the individual strain (with replicable determinations), species, genus, and overall set for determination of test method equivalences and/or inter-laboratory consistancy.

Computer graphic algorithms are being tested to aid microbiologist in visualizing individual similarities as well as hierarchical group memberships among strains.

PHS-6040
(Rev. 2-81)

LABORATORY OF BIOCHEMISTRY

A major change occurred in the Laboratory of Biochemistry with the departure of Dr. Karl A. Piez, Chief, Laboratory of Biochemistry, and Section Chief of the Protein Chemistry Section, effective February 26, 1982 after more than 30 years of service. He is continuing active direction of research in the biotechnology industry. Dr. Piez established the Protein Chemistry Section in 1961, when Dr. Frank McClure was Laboratory Chief, and assumed responsibility for the Laboratory in 1967. His outstanding, highly regarded research in the area of collagen biochemistry and his effective skill in recruiting investigators and in managing the Laboratory of Biochemistry were crucial for establishing the Laboratory as an internationally renowned research center in the biochemistry of connective tissues. During most of the time since the departure of Dr. Piez, the Chief, Proteoglycan Chemistry Section, has assumed responsibility for maintaining the administration of the Laboratory.

As discussed in last years report, the Laboratory of Biochemistry contains three Sections, the Proteoglycan Chemistry Section, the Enzyme Chemistry Section and the Protein Chemistry Section all of similar size and composition. Laboratory personnel total about 25 with a ratio of research to support staff of about 2:l and a ratio of temporary (postdoctoral and visitors) to tenure research staff of about 2:l. Of the temporary research staff, about two-thirds are supported by mechanisms (postdoctoral fellow ships, expert position) that do not count against our position ceiling, a necessity for maintaining program vitality in the presence of declining budgets for intramural research.

There were no major changes in the sizes of the research programs, which continue to be limited by our position ceiling, our ability to recruit through other mechanisms, and the available space. The programs as before collaborated extensively with scientists inside and outside of NIH, an indication of the strength of the research and of the great interest it has attracted outside the Laboratory. Such collaborative efforts which extend program interests into areas of expertise not available within the Laboratory are a valuable source of synergistic research efforts and of enhanced overall productivity. The Laboratory continued to utilize some space in building 2 (NIADDK), where the nuclear magnetic resonance (nmr) instrumentation is located, and the electron microscope facilities in the Laboratory of Biological Structure, NIDR. Space considerations remain a source of concern. The programs in the Laboratory of Biochemistry have always been crowded, and it is imperative for the maintenance of program productivity, morale and vitality that a proposed reorganization of space be carried out quickly and efficiently in order to minimize any adverse effects on program momentum.

The unifying theme of the Laboratory of Biochemistry has been its focus on original research in areas of biochemistry, molecular structure and function of normal, diseased and repairing connective tissues. The senior personnel participate directly in research and in a variety of other important professional activities; including participation in national and international meetings, serving as editors on journals, reviewing manuscripts for a wide variety of scientific journals, reviewing grant applications for granting agencies and foundations, teaching in the Graduate School at NIH, providing seminars to professional programs inside and outside of NIH, serving on review committees, and organizing research meetings. As was also noted in last year's report, management responsibilities have continued to consume time and effort of Laboratory personnel, especially in administrative aspects for travel and review of personnel. Such efforts inevitably detract from the primary purpose of the Laboratory which is to conduct creative and current research in important biomedical research areas.

PROTEOGLYCAN CHEMISTRY SECTION

Proteoglycans are complex macromolecules which contain glycosaminoglycans and other oligosaccharides covalently attached to distinct core proteins. They are critical structural elements of connective tissue. As examples: (a) cartilage proteoglycans directly influence the shape of the developing skeleton, and they provide the resiliency and resistence to compressive load required for proper physical function in adult cartilages; (b) corneal proteoglycans are essential components for maintaining the normal, highly organized matrix of the stroma and for transparency of the tissue; (c) proteoglycans are important constituents of basement membranes and serve as a filtration barrier in kidney and are essential for morphogenesis in branching epithelial systems in the developing salivary gland; (d) proteoglycans are involved in ovarian follicular maturation leading to ovulation where their synthesis is under hormonal control.

The Proteoglycan Chemistry Section continues to study the structure and biosynthesis of proteoglycans from cartilage and other tissues. (a) The core protein precursor, which is processed to the completed proteoglycan by addition of chondroitin sulfate chains in the Golgi complex, has been localized to the rough endoplasmic reticulum compartment by cell fractionation studies using chondrocytes from the rat chondrosarcoma. This precursor, with an apparent molecular weight of ~350,000, has an intracellular half

life of 60-90 minutes, and it contains xylosylated serines indicating that the first, and perhaps regulatory, step in chondroitin sulfate synthesis occurs long before the remainder of the chains are added. (b) Organ cultures of bovine articular cartilage have been developed to study the regulation of proteoglycan synthesis by chondrocytes maintained within a nearly normal surrounding extracellular matrix. Bacterial lipopolysaccharides have been shown to increase turnover as well as to inhibit synthesis of proteoglycans in a dose-dependent and reversible manner. Such endotoxins can have profound effects on the function of normal and osteoarthritic articular cartilages as can normal metabolic regulators of cartilage tissue maintenance. (c) Granulosa cells in culture synthesize several distinct classes of dermatan sulfate and heparan sulfate proteoglycans. Several new methodologies, which have wide general applicability were developed for separating and characterizing these proteoglycans. The synthesis of one class of dermatan sulfate-proteoglycan was shown to be markedly stimulated by such reproductive hormones as luteinizing hormone and follicle stimulating hormone. One class of heparan sulfate proteoglycan has been shown to be intercollated into membranes, probably as an integral, cell surface membrane component. (d) The complete chemical structures of the high mannose-oligosaccharides and of the keratan sulfate linkage region of the corneal keratan sulfate-proteoglycans have been determined. These structures clearly establish the relationship of this proteoglycan with normal glycoprotein structure and biosynthesis. (e) A chondroitin sulfate-proteoglycan synthesized by aortic smooth muscle cells has been extensively characterized. This proteoglycan is closely related to, but not identical with the major class of cartilage proteoglycans. It has a hyaluronic acid-binding region and forms link protein stabilized aggregates, but has far fewer chondroitin sulfate chains and far more O-linked oligosaccharides. The identification of this proteoglycan in aortic tissues extends the importance of this class of proteoglycan to a non-cartilaginous connective tissue.

ENZYME CHEMISTRY SECTION

The Enzyme Chemistry Section continues to study transglutaminase, an enzyme which catalyzes ϵ-(γ-glutamyl)lysine crosslinks. It is now evident that these enzymes catalyze many important biological reactions including: formation of crosslinks directly between protein molecules as in fibrin stabilization, cross-bridging of protein molecules through polyfunctional amines, and incorporation of biogenic amines into specific cellular proteins. Thus, the role of transglutaminases in regulatory processes may be of critical importance. Further, the evidence that these enzymes promote crosslinks between extracellular matrix molecules such as collagen, fibronectin, and fibrin and membrane molecules such as actin and myosin indicates the importance for continuing basic studies on this class of enzymes.

Three broad aspects of the problem continue to be studied: (a) structural details of the substrates for the enzyme and their effect on the reaction mechanism; (b) biological roles for transglutaminases and crosslinks; (c) regulation of transglutaminase during liver regeneration and tissue repair.

Major findings for (a) include: About 40 peptides with variations in sequence around glutamine 167 of β-casein were prepared and studied as each side of the glutamine are important for specificity and that a critical lysine residue can only be replaced by certain hydrophobic amino acids. This study has provided several excellent new substrates and has suggested that the tertiary structure of the substrate may be a critical detetrminant for specificity. Photolabile, bifunctional amino substrates were synthesized. Transglutaminase was then used to crosslink these substrates and thereby prepare photosensitive derivatives of several peptide hormones (substance P, glucagon I-6, calcitonin) for subsequent receptor studies.

Major findings for (b) include: Crosslinks between proteins and polyamines have been identified following clotting of rat seminal fluid, and putrescine and spermidine were found to be γ-glutamyl linked to proteins following mitogen stimulation of peripheral lymphocytes. Both of these biological systems appear to be mediated by transglutaminases indicating the wide biological roles these enzymes may play. A cyclotransferase, which catalyzes the breakdown of the crosslink through a cyclization of the glutaminyl group of the crosslink was partially purified from rabbit kidney. Evidence for the involvement of this enzyme in the normal catabolism of the crosslinks was obtained.

Major findings for (c) include: The concentration of membrane associated transglutaminase activity increased 5-7 fold following partial hepatectomy of rats even though total enzyme concentration (primarily cytosolic) decreased. This suggests that membrane bound transglutaminase may play a role during cell division and liver regeneration. Rabbits with an experimentally-induced Factor XIII (transglutaminase) deficiency did not produce an endotoxin-mediated inflammation suggesting that this enzyme may play a role in the inflammatory process. A single glutamine residue in a fast reacting plasmin inhibitor (α_2-PI) was modified by Factor XIII. The modified molecule was still an effective inhibitor of plasmin and could be

crosslinked into a region near the C terminus of fibrin in the presence of Factor XIII. These studies help define the crosslink patterns and the role of transglutaminase in regulation of fibrin clot formation and its subsequent degradation.

A new project, first described in last year's report, concerns the synthesis of an unusual amino acid, hypusine. Evidence indicates that it is formed on a specific low molecular weight, cytosolic protein by posttranslational modification of specific lysine residues by transfering a butylamine moiety from spermidine followed by oxidation of a specific CH_2 group. The precursor-protein exists in resting lymphocytes where it is continuously turned over. Formation of hypusine on this precursor occurs only after the cells are mitogenically stimulated suggesting a possible role for the hypusine-protein in cell division.

PROTEIN CHEMISTRY SECTION

The Protein Chemistry Section continues to work on the molecular structure, packing and interactions of major connective tissue macromolecules, primarily collagen and proteoglycans, with emphasis on how macromolecular parameters influence organization and function of connective tissues. Studies continue on the mechanism of fibril formation by different collagen types, and on the molecular dynamics of collagen and proteoglycans as determined by ^{13}C nuclear magnetic resonance (nmr) methods. Further, ^{13}C-nmr is being used to study gelation of hemoglobin S (sickle cell) in erythrocytes.

The ability of lathyritic type II (cartilage) collagen to assemble into fibrils was studied under defined conditions *in vitro*. Native banded, large diameter fibrils formed as determined by kinetic measurements and electron microscopy. The fibrils differed significantly from the narrow diameter fibrils observed *in vivo* suggesting that the mechanisms for collagen fibril formation in cartilage involve additional factors. The presence of cartilage proteoglycans during assembly *in vitro* did not alter final fibril morphology for either lathyritic type I (tendon) or type II collagen suggesting that final fibril architecture *in vivo* may not be directly attributable to proteoglycans. Additional studies indicate that the aldehydes in normal type I collagen influence assembly kinetics considerably when compared with lathyritic type I (without aldehydes) suggesting that aldehydes participate more directly in fibril assembly than previously thought. Initial studies on vitamin D deficient rats indicate that the skeletal type I collagen is deficient in crosslinks whereas skin type I collagen is not.

The methodology for nmr spectroscopy has advanced significantly during the past years permitting studies on the mobility of molecular groups in immobile as well as mobile samples ranging from pure macromolecules in various solvent conditions to macromolecules directly in tissue. Two pulsed nmr spectrometers were built to provide solid state spectra of ^{2}H, ^{13}C and ^{31}P, extending the capabilities of this technology greatly for studies on connective tissues, including mineralized tissues such as bone and dentin. A series of experiments were done in which specific ^{2}H- or ^{13}C-labeled amino acids were used as precursors *in vivo* or in organ culture to enrich the abundance of these isotopes in collagen in specific tissues. Backbone mobility of collagen in intact (mineralized) calvaria was significantly less than in demineralized calvaria. Backbone mobility was absent at -35°C. These results suggest that mobile water is required for collagen mobility and that replacement of mobile water by mineral (or ice) effectively eliminates mobility of the collagen backbone. NMR measurements on alanine crystals indicate that the rotation of methyl groups is reduced by two orders of magnitude in the crystal structure, suggesting that measurements of methyl rotation rates can provide information about packing of methyl groups within protein structures. This technique is being applied to other amino acid crystals. Compression of cartilage is accompanied by water loss from the domain of proteoglycan molecules which should lead to alterations in glycosaminoglycan mobility. An investigation of the effect of dehydration and of selective counterion interactions on proteoglycan mobility as assessed by nmr parameters is underway. Solid state ^{13}C-nmr was used successfully to estimate the polymer fraction of the hemoglobin in SS (homozygous sickle) erythrocytes. In SS cells polymer was detected at high oxygen saturation ($> 90\%$). In contrast, in AS (heterozygous sickle) cells, polymer was detected only when oxygen saturation fell below 70%. These results are consistent with the absence of pathology in individuals having AS cells. Such studies will be useful for investigating the mechanism of gelation within intact erythrocytes and for evaluating the effects of potential inhibitors.

CONCLUDING REMARKS

All of the programs in the Laboratory have been active and productive. The absence of Dr. Karl Piez has posed major problems for administering the programs and many minor problems for continuing research activities at the customary, unabated pace. However, to a large extent, the momentum of the Laboratory remains unchanged. Thus there is optimism that the major research efforts will continue to be productive, and hopefully they will emerge from this time of change with stronger goals and identification within the needs of the intramural research programs at NIDR.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00001-30 LB

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Transglutaminases: Specificities, Physiological Functions, and Catabolism of Products

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Folk, J. E.	Chief, Enzyme Chemistry Section	LB	NIDR
Fink, M. L.	Staff Fellow	LB	NIDR
Park, M. H.	Visiting Fellow	LB	NIDR

COOPERATING UNITS (if any)

Dr. Jeffrey J. Gorman, University of Melbourne, Parkaville, Victoria, Australia

LAB/BRANCH: Laboratory of Biochemistry

SECTION: Enzyme Chemistry Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.75	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the specificity and catalytic mechanism of transglutaminases are underway. The cellular and extracellular functions of the products of transglutaminase action are under investigation. Knowledge has been obtained as to the catabolic fate of these transglutaminase products.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00002-32 LB

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structural Studies on Collagen

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Piez, Karl A.	Chief, Protein Chemistry Section	LB	NIDR

COOPERATING UNITS (if any)

Dr. Benes L. Trus, DCRT; Dr. Michael Beer, Johns Hopkins University; Dr. Joseph Wall, Brookhaven National Laboratory.

LAB/BRANCH: Laboratory of Biochemistry

SECTION: Protein Chemistry Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of this project is an understanding of collagen structure from the molecular to the fibril level. Emphasis has been on conventional and scanning transmission electron microscopy and analysis of micrographs by computer methods. During the current year research has shifted to fitting of models to x-ray diffraction data based on a new unit cell. We have shown that the five-stranded microfibril model, if compressed to place collagen molecules in cross sections on a near-hexagonal lattice, fits positional and intensity data obtained by x-ray diffraction of rat tail tendon collagen fibers.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00049-11 LB

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Physiological Role of Transglutaminases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Chung, S. I.	Research Chemist	LB	NIDR
Chang, S. K.	Visiting Fellow	LB	NIDR
Carmassi, F.	International Fellow	LB	NIDR

COOPERATING UNITS (if any)

Dr. Mark Lewis, SEI; Dr. Dennis Gelanakis, SUNY, NY; Dr. Soo Young Lee, Catholic Medical School, Seoul, Korea

LAB/BRANCH: Laboratory of Biochemistry

SECTION: Enzyme Chemistry Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.55	2.80	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The physiological function and the mode of regulation of the transglutaminases are being studied including their role in the modulation of specific cellular processes and in fibrio-connective tissue matrix stabilization during tissue repair. A novel form of transglutaminase, distributed in cell membranes and nuclei, has been isolated and characterized. This membrane-associated transglutaminase, which is present as an inactive form in resting cells, is one of the first enzymes to be activated during cell stimulation and proliferation.

The physiological significance and biochemical mechanism of Factor XIIIa (plasma transglutaminase)-catalyzed crosslinking of fast-reacting plasmin inhibitor (α_2-PI) to fibrin and other matrix proteins is under investigation both in vivo (Shwartzman's phenomenon) and in vitro. Fibrin crosslinked to α_2-PI has been shown to be resistant to plasminolysis.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZD1 DE-00134-08 LB

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Biosynthesis of Proteoglycans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Hascall, V. C.	Chief, Proteoglycan Chemistry Section	LB	NIDR
Yanagishita, M.	Special Expert	LB	NIDR
Fellini, S.A.	NIH Postdoctoral Fellow	LB	NIDR
Stevens, J. W.	Arthritis Foundation Fellow	LB	NIDR
Morales, T.	Kroc Foundation Fellow	LB	NIDR
De Luca, S.	Guest Researcher	LB	NIDR

COOPERATING UNITS

C. Handley, Monash Univ., Australia; J. Kimura and E. Thonar, Rush-Presbyterian-St. Lukes Medical Center, Chicago; L. Rosenberg, Montefiore Medical Center, NYC; A.R. Poole, Shriner's Children's Hosp., Montreal; T. Wight, Univ. of Washington, Seattle; S. Nilsson, NCI; K. Nakazawa and D. Newsome, NEI; D. Beebe, Armed Forces Medical School, Bethesda; Y. Chang, Fu-wai Hospital, Peking, China; L. S. Lohmander, University of Lund, Lund, Sweden.

LAB/BRANCH: Laboratory of Biochemistry

SECTION: Proteoglycan Chemistry Section

INSTITUTE AND LOCATION: NIDR, NIH Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.00	6.00	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to study the chemical and physical properties and the biosynthesis of proteoglycans in a number of tissues. Topics of present interest include: 1) Protein chemistry of the hyaluronic acid-binding region of proteoglycans from the Swarm rat chondrosarcoma, 2) biosynthesis of the proteoglycan core protein precursor and its associated oligosaccharides and of the link protein by chondrocytes, 3) biosynthesis of proteoglycans by ovarian granulosa cells, 4) biosynthesis of proteoglycans by aortic smooth muscle cells, 5) characterization of corneal stroma proteoglycans, 6) characterization of oligosaccharides and keratan sulfate in fetal epiphyseal cartilage, 7) regulation of biosynthesis and turnover of proteoglycans in cultures of bovine articular cartilage, 8) development of highly sensitive and specific assays for hyaluronic acid.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00157-07 LB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biophysical Studies on the Structure of Connective Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Torchia, D. A.	Biophysicist	LB	NIDR
Batchelder, L. S.	Staff Fellow	LB	NIDR
Sarkar, S. K.	Visiting Fellow	LB	NIDR

COOPERATING UNITS (if any)
Dr. A. N. Schechter, NIADDK; Dr. C. H. Niu, NIADDK; Dr. J. V. Silverton, NHLBI

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Protein Chemistry Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.25	3.00	2.25

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the molecular structure of fibrous proteins and proteoglycans, and to study intracellular gelation of Hemoglobin S. The structural information obtained will be correlated with function. Areas of present interest are 1) Molecular structure and dynamics of collagen. Carbon-13 and deuterium magnetic resonance techniques are being used to study the structure and interactions in collagen fibers. 2) Proteoglycan dynamics. Carbon-13 magnetic resonance is also being used to study the molecular mobility of the polysaccharide and protein chains in cartilage proteoglycans. 3) Carbon-13 magnetic resonance is being used to study the extent and mechanism of hemoglobin S gelation in erythrocytes. For these studies, magnetic resonance spectrometers have been assembled which give multinuclear spectra of solids. High power decoupling, cross-polarization, magic angle spinning, and solid echo experiments are all performed.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00215-06 LB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Connective Tissue: Formation and Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Lee, S. L.	Staff Fellow	LB	NIDR
Piez, K. A.*	Chief	LB	NIDR

*Retired from government service 2-26-82

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Protein Chemistry Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.00	1.25	1.75

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range goal of this project to study interactions between connective tissue macromolecules as a way to understand connective tissue formation and structure. The topics of study are: 1) The mechanism of collagen fibril formation in vitro; 2) the role of non-collagenous macromolecules in collagen fibril architecture, and 3) the role of vitamin D metabolites in collagen fibril crosslinking in vivo. Previously, this laboratory developed a reproducible in vitro fibril assembly system and showed that type I collagen from rat tail tendon assembled into fibrils via a multistep process. This system is being used to study lathyritic Type I, lathyritic type II, and type III collagen assembly in vitro both in the presence and absence of proteoglycans and lysyl oxidase. The role of vitamin D metabolites in collagen crosslinking is being evaluated in vivo using vitamin D-deficient rachitic rats.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00311-02 LB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Unusual Amino Acid, Hypusine: Mechanism of Formation and Function in Cellular Protein.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Polk, J. H.	Chief, Enzyme Chemistry Section	LB	NIDR
Park, M. H.	Visiting Fellow	LB	NIDR

COOPERATING UNITS (if any)
Dr. H. L. Cooper NCI, LPP

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Enzyme Chemistry Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	.50

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The amino acid hypusine has been identified in the same single low molecular weight protein in numerous mammalian cells. Evidence has been accumulated for its posttranslational formation from lysine and the butylamine moiety of the polyamine, spermidine, followed by hydroxylation. The findings demonstrate a novel polyamine metabolic pathway.

PHS-6040 (Rev. 2-81)

LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY

In November, 1981 the Laboratory was reviewed by the NIDR Board of Scientific Counselors and *ad hoc* review panel of specialists in microbiology and immunology. One of the major recommendations made by the review group was that a mechanism be developed and implemented for the systematic and expeditious replacement of key independent investigators who leave. It became obvious to this panel that the loss of key personnel over the past few years without full replacement has represented serious restraints on the continuity of programs and on our progress. Nevertheless, the Laboratory has compensated to a certain degree by the use of staff fellows, the Visiting Fellow program and by recruiting through postdoctoral fellowship award mechanisms. The Laboratory is also deficient in full time technical support personnel. However, it is also to the credit of our senior investigators who have spent considerable time and effort to provide some relief from this situation by recruiting and training students in various categories of part time employment. Despite these problems, notable progress was achieved in our multidisciplinary research projects. The more important findings are summarized below.

MICROBIOLOGY SECTION

The Microbiology Section continues its ecological, biochemical and molecular biological studies on members of the oral microbial flora. Our ecology program has focused on a highly specific form of cell-cell recognition between strains of *Streptococcus sanguis* and certain species of *Actinomyces* that results in the formation of large macroscopic coaggregates. Some of these interactions are mediated by complementary surface components composed of a lectin on one cell type and carbohydrate receptor on the other cell type. The importance of this phenomenon in *in vivo* plaque development was indicated by a prior comprehensive survey which demonstrated that fresh human oral isolates participated in the same coaggregation patterns established with stock laboratory strains. Further evidence for the *in vivo* significance of coaggregations has come from the recent demonstration that the reactions occur in human saliva as well as in buffer. Moreover, those coaggregations that are reversed by lactose in buffer also show lactose reversibility in saliva. As indicated, certain of the intergeneric coaggregations are reversed by lactose and these have been studied collaboratively with investigators in the Humoral Immunology Section. Others, however, are insensitive to lactose and these have received considerable current attention. In an effort to gain insight into the nature of the cell surface structures involved in these interactions, a genetic strategy has been employed using various coaggregation-defective mutants of *S. sanguis*. Results from these studies have revealed that one type of lactose-insensitive coaggregation that occurs betweeen *S. sanguis* and *A. naeslundii* was inhibited by N-acetylneuraminic acic (NANA). It appears, therefore, that *S. sanguis* possesses a NANA-sensitive surface lectin and that NANA or a structurally related compound is a surface component of *A. naeslundii.* Another approach to studying these surface components has involved the use of bacteriophage resistant mutants of *Actinomyces viscosus.* These mutants as well as the wild type strain participated in the lactose-inhibitable coaggregations. However, unlike the parental strain, the mutants had lost the capacity to react in certain of the lactose-insensitive reactions. The bacteriophage resistant mutants are thus a new and potentially powerful tool for resolving the nature of the lactose-insensitive surface structures.

The Section's biochemical studies have centered on the mechanism of carbohydrate transport and metabolism and the turnover of cellular proteins in the lactic acid bacteria. Among the important advances in this program over the past year has been the resolution of the mechanism by which 2-deoxyglucose inhibits the growth of *Streptococcus lactis* and certain other sensitive streptococci. The glucose analogue was found to be transported into the cell by a phosphoenolpyruvate glucose: phosphotransferase system and was then rapidly dephosphorylated by an intracellular phosphatase and exported as free 2-deoxyglucose. The net result of this complex series of reactions is the operation of a futile cycle which functions to deplete the cell of energy (ATP). This study is providing important new information potentially relevant to the selection or design of sugar analogues that might be used to restrict the proliferation of oral streptococci implicated as etiological agents of dental caries.

Significant advances have also been made in studies oriented toward resolving the pathway of xylitol metabolism in certain of the lactic acid bacteria. Two of the three enzymes induced specifically for the utilization of this pentitol have now been purified to electrophoretic homogeneity and extensively characterized. These are the soluble xylitol transport component, Enzyme III, and the NAD-linked xylitol-5-phosphate dehydrogenase. Both of these enzymes have been found to contain covalently bound lipid and this unusual property is now being analyzed to determine whether it is linked to specific catalytic or regulatory functions of the proteins.

Another facet of the biochemical studies is concerned with mechanisms by which the bacterial cell recognizes and processes abnormal or nonfunctional proteins. *Streptococcus salivarius* produces a cell-associated fructosyltransferase (FT) that is rapidly inactivated during the lag phase of growth. We have now demonstrated that FT inactivation is a two step phenomenon. The first step is a redox reaction that requires a reduced pyridine nucleotide (NADH or NADPH), Cu^{++}, and a phospholipid. This step results in a loss of FT catalytic activity. The data indicate that this reaction modifies the enzyme in such a way that it becomes a substrate for a protease which, in a second step, then degrades the protein. Present evidence suggests that the redox enzyme system and probably the protease as well are normally located in the cell membrane.

For the past several years, molecular biological approaches have been employed in our studies on microbial physiology and metabolism. This approach, as applied to work on lactose metabolism in *Lactobacillus casei*, has been particularly fruitful over the past year. We had found previously that lactose metabolism in *L. casei* is a plasmid associated trait. Both the lactose transport components and phospho-β-galactosidase (P-β-gal) are encoded on a 23 Mdalton plasmid (pLZ64). This finding has provided an opportunity to study the function, structure and regulation of genes in *L. casei*. To this end, pLZ64 was cloned into *Escherichia coli* using the plasmid vectors pBR322 nd pACYC184 and the restriction enzymes Hind III, Pst I, BamH I and EcoR I. A large clone bank has now been established for use in a variety of future studies. One clone ws found which expressed P-β-gal activity and it has been analyzed in some detail. This transformant carried a 7.9 Kpb Pst I B fragment of pLZ64 DNS inserted into the single Pst I site of the vector of pBR322. The tranformant contining the recombinant plasmid (pLZ600) produced a P-β-gal that was identical in physical and kinetic properties to that synthesized by *L. casei* 64H carrying pLZ64. Minicell analysis of transformants containing various subclones has been used to determine both the position and direction of transcription of the P-β-gal gene. The development of this technology offers the exciting potential for studying a number of biochemical traits exhibited by the lactic acid bacteria at the molecular level. We are now exploiting this system, for example, with attempts to clone a tetracycline (Tc) resistance plasmid from a strain of *Streptococcus mutans*. If successful, this would allow the accumulation of sufficient material to compare, at the level of DNA sequence homology, the *S. mutans* Tc determinant to Tc determinants carried by other microorganisms. Such information can give insight into the possible origin and extent of transmissibility of this trait among various bacteria.

CELLULAR IMMUNOLOGY SECTION

The Cellular Immunology Section is investigating basic mechanisms by which host defenses to microbial and other antigens mobilize and modulate cellular and antibody-mediated inflammatory reactions. A major effort involves the study of hormone-like immunoregulatory factors produced by inflammatory cells. Both the biological effects and biochemical characteristics of a number of these mediators are being intensively investigated. These mediators which are produced by stimulated monocytes, lymphocytes, growing keratinocytes or cell lines are produced in small amounts and are active at 10^{-T116} to 10^{-T115} concentrations which complicates the biochemical purification.

Current investigations have revealed that some of these mediators have a multiplicity of biological effects either by activating a variety of target cells directly or by initiating a cascade of mediator-cell interactions that amplify inflammatory reactions. The mechanism of action of Interleukin 1 (IL 1) produced by macrophages involves both of these pathways as follows: IL 1 acts directly on thymocytes to augment their proliferative response; IL 1 is directly mitogenic for fibroblasts and promotes their production of prostaglandins and fibronectin; *in vivo* administration of IL 1 induces hepatocyte production of an acute phase protein, serum amyloid A (SAA) and stimulates cells of the hypothalamic fever center to produce prostaglandin which results in a febrile response; and finally, IL 1 can chemotactically attract as well as activate polymorphonuclear and mononuclear leukocytes. IL 1 also indirectly amplifies immunological reactions by participating in several cascades as follows: Macrophages are stimulated by a multiplicity of agents including mediators produced by lymphocytes and fibroblasts e.g. (colony stimulating factors, CSF) to produce IL 1. IL 1 in turn promotes the production by lymphocytes of Interleukin 2 (IL 2) as well as other lymphokines with chemotactic, macrophage activating and lymphocytotoxic properties. IL 2 itself has a number of immunological effects such as supporting the growth of cytotoxic lymphocytes and natural killer cells and promoting interferon production by T lymphocytes and antibody production by B lymphocytes. The cascade of mediators leading to antibody production also involves the participation of a B cell growth factor and a T cell replacement factor that promotes the growth and differentiation of B and T lymphocytes respectively. The immune interferon also has a variety of anti-proliferative and differentiating effects that promote natural killer and cytotoxic lymphocytes functions and promote the accessory cell capabilities of macrophages in antigen activation of lymphocytes.

It should also be mentioned that an IL 1-like mediator is produced by keratinocytes and by corneal and oral mucosal epithelial cells. Presumably the production of these mediators in response to callenging exogenous stimuli, irritants or injurious agents participates in promoting local as well as systemic host defense and reparative processes. In concert with this conclusion is the finding that an IL 1 like factor is present in human gingival fluid. In fact, it is present to a greater extent in gingival fluid obtained from sites manifesting gingivitis. These findings indicate that human gingival fluid contains thymocyte growth factor(s) which may amplify immune and non-immune reactions in human periodontal tissues.

Collaborative studies with the Clinical Immunology Section designed to produce monoclonal murine hybridoma-derived antibodies to some of the mediators are continuing, but are proving difficult. A number of productive clones have proven to be unstable and have been lost. In addition, the considerable effort and expertise needed for these studies have been difficult to obtain due to high turnover of staff and inexperienced personnel.

In addition to performing laboratory studies, members of the Cellular Immunology Section have been engaged in organizing, chairing and participating in the 3rd International Workshop on Lymphokines. This effort is designed to promote communication and progress in this important area of study.

HUMORAL IMMUNITY SECTION

Investigations in the Humoral Immunity Section have provided important insights into the immunological mechanisms involved in the destruction as well as the hypertrophy of connective tissue. The *in vitro* findings that a variey of inflammatory inciting agents activate lymphocytes and macrophages to secrete enzymes and mediators which degrade collagen, initiate the proliferation of fibroblasts and stimulate collagen production by these cells are being further defined and extended to animal model systems and human disease states.

The activation pathway leading to the production of collagenase by macrophages *in vitro* involves initial stimulation, prostaglandin E_2 synthesis and elevation of cAMP. Recent findings indicate that an additional step in this sequence may be the participation of the ornithine decarboxylase pathway, perhaps via its production of polyamines since agents which inhibit prostaglandin synthesis block both ornithine decarboxylase and collagenase production and specific inhibition of ornithine decarboxylase also blocks the production of collagenase.

The immune system has been found to contribute to abnormal connective tissue metabolism in several animal model systems. In osteopetrotic (op) rats, in which defective bone resorption can be cured by spleen or bone marrow transplantation from normal syngeneic rats, both thymocyte and macrophage defects have been defined. The proliferative responses of the thymocytes to several stimulants are exceptionally low. Further exploration of the thymocyte populations has revealed that a subset of these cells, obtained by counterflow centrifugal clutriation, does proliferate well. These findings suggest that an abnormally active suppressor cell population has been removed; a concept which will be further explored by cell mixing experiments. Immunological defects in lymphocytes and macrophages have also been identified in rats which have developed rickets due to Vitamin D deficiency. The development of arthritis in genetically susceptible strains of rats by streptococcal cell walls appears to be related to the immune status of these animals. Lymphocyte proliferation, lymphokine production, macrophage prostaglandin production and monokine synthesis are suppressed in both susceptible and resistant strains after the administration of the cell wall preparation. However, the susceptible rats recover immune function earlier suggesting that normal immune function is essential to the development of the arthritic inflammation.

The immune status of patients with connective tissue disorders has also been assessed by collaborative studies with scientists at NIADDK. Prior to treatment of rheumatoid arthritis by leukopheresis, patients can be grouped into two general categories: Those that exhibit normal immune function and those expressing depressed immunological responses. It has been found that the clinical status of the patients with suppressed responses improves following leukopheresis and that their cellular responses are restored during the course of this treatment. Lymphopenia is not induced in these patients and the beneficial effects are currently attributed to depletion of a functionally abnormal subset of mononuclear cells. Of major interest is the recent finding that lymphocytes and monocytes isolated from inflamed synovial tissue of fluid of rheumatoid arthritis patients spontaneously release a mediator(s) which is mitogenic for fibroblasts. These studies provide evidence that the cellular derived mediators which can be induced *in vitro* are produced *in vivo* during the course of chronic inflammatory disease processes.

The identification and characterization of microbial surface components which are involved in adherence to other bacteria and mammalian cells continue to be

areas of major interest in this Section. As indicated previously, a strong and productive collaborative effort in this area exists between investigators in this Section and the Microbiology group. Clearly emerging from these studies is the finding that distinct functions can be assigned to specific bacterial surface structures. Two types of fimbriae (Ag1 and Ag2) have been identified on *Actinomyces viscosus* T14V which differ in their functional properties. Those designated as Ag2 are associated with a lactose sensitive lectin activity that mediates coaggregation with certain oral streptococci and adherence to mammalian cells. The Ag1 fimbriae, which lack lectin activity, interact with saliva coated hydroxyapatite. These functional distinctions have been defined by the use of monoclonal and monospecific antibodies and their Fab fragments as well as by the recent isolation of mutants which lack the Ag1, the Ag2 or both fimbriae. The concept that separate and specific surface structures are involved in bacterial adherence to different surfaces within the oral environment has been further examined by the immunochemical evaluation of several strains of *A. viscosus* and *A. naeslundii*. Both types of fimbriae were detected on all strains of *A. viscosus* by agglutination reactions. In contrast, all the strains of *A. naeslundii* were agglutinated by Ag2 antibodies but several were not agglutinated by the Ag1 antibodies and additional studies with these latter strains failed to reveal Ag1 fimbriae. These findings correlated well with established differences in the oral distribution of these bacteria, particularly the greater ability of *A. viscosus* to attach to and colonize tooth surfaces and the preference of typical strains of *A. naeslundii* for certain oral epithelial surfaces.

CLINICAL IMMUNOLOGY SECTION

Studies in the Clinical Immunology Section are continuing with monoclonal antibodies to a number of different antigens. Such reagents are useful tools for dissecting different functional-structural domains in molecules. A series of hybridomas have been produced which react with the immunoglobulin E receptor on the membrane of mast cells and on the rat basophilic leukemia cells. These series of antibodies distinguish the site at which the immunoglobulin binds (the actual receptor site) from the neighboring sites on the receptor molecule. Other hybridomas bind to the parts of the receptor which are in the membrane. Thus, the different domains of the receptor are being mapped with these monoclonal antibodies. A similar approach has been used to study the immunoglobulin E molecule. A series of hybridomas have been prepared which react with different domains of the molecule. For example, some of these are to sites which are hidden (or not available) when the IgE molecule is in its receptor on the cell surface. These experiments will help us understand the mechanisms of cell activation.

Studies are continuing on the mechanisms of cell secretion. In the past few years the rat basophilic leukemia cell line has become very useful for defining the biochemical changes which occur during histamine release. The cross-linking of the IgE molecule results in increased phospholipid methylation, Ca^{2+} influx and the release of arachidonic defects at different steps in the secretory process: these mutants allow us to determine the sequence of biochemical steps. We have also introduced chromosomal markers into these cells and can use them for cell hybridization experiments. With such studies a number of complimentation groups have been defined. The experiments are now aimed at better definitions of the phospholipidase activation steps involved in the release process.

LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00007-22 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Regulation of Carbohydrate Metabolism in Oral Bacteria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Wittenberger, Charles L.	Research Microbiologist	LMI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Microbiology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.00	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pathways of carbohydrate metabolism operative in oral bacteria and mechanisms by which cellular metabolism is regulated continue to be under investigation. Current emphasis is placed on delineating the mechanism underlying the inactivation of a cell-associated fructosyltransferase (FT) produced by Streptococcus salivarius. Our present data point to the fact that FT inactivation is a two step phenomenon. The first step appears to be an oxidation-reduction reaction that requires a reduced pyridine nucleotide (NAD(P)H), Ca^{++}, and a phospholipid or detergent. Such requirements are also exhibited by certain microbial and mammalian liver mixed function oxidases. This step results in a loss of FT catalytic activity. We propose that this reaction modifies the enzyme and "marks" it as a substrate for a protease which, in a second step, then degrades the protein.

PHS-6040 (Rev. 2-81)

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00022-16 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Comparative Physiology of Lactic Acid Bacteria and Other Oral Microbes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

London, Jack F.	Research Microbiologist	LMI NIDR
Rolenbrander, Paul E.	Senior Staff Fellow	LMI NIDR
Kagermeier, Angelika	Visiting Fellow	LMI NIDR

COOPERATING UNITS (if any): R. Celesk, Dept. of Biology, Univ. of Ohio at Dayton; N. Neimark, Downstate Med. Ctr., SUNY, Buffalo

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Microbiology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	2.25	1.00

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two of the three enzymes responsible for the catabolism of xylitol by Lactobacillus casei, namely the transport component Enzyme III^{Act} and xylitol-5-phosphate dehydrogenase, have been purified to electrophoretic homogeneity and are presently being characterized. Both enzymes are unusual in that they contain significant amounts of lipid. Antisera have been prepared against the two enzymes and are being used as probes to (1) detect and measure structural homology between isofunctional counterparts in other bacteria and (2) to completely characterize the genetic lesions in our large catalogue of mutants. Adherence studies with gram negative oral bacteria have been extended to include strains of Actinobacillus actinomycetemcomitens. Like the oral Cytophage strains described in our earlier studies, actinobacilli are capable of adsorbing to spheroidal hydroxyapatite (SHA) in comperatively high numbers. Serum and saliva treatment of the SHA inhibited adsorption. Treatment of the cells with various proteases, phospholipases and neuraminidase provided a means of distinguishing between strains based on binding capabilities.

PHS-6040 (Rev. 2-81)

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00034-14 LMI

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Histamine Release

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Siraganian, R.	Chief, Clinical Immunology	LMI NIDR
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Basciano, L.K.	Microbiologist	LMI NIDR

COOPERATING UNITS (if any)

NIAMDD, Arthritis & Rheumatism Branch, NIH
NCI, Laboratory of Cell Biology NIH
NCI, Laboratory of Theoretical Biology, NIH
NIMH, Laboratory of Clinical Science, NIH

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Clinical Immunology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.50	3.00	.50

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflammation. Among the histamine releasing agents employed are IgE antibody, the anaphylatoxins, and the Ca^{2+} ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and changes in phospholipid methylation during cell activation. Large number of cells can be obtained for biochemical studies and biochemical variants selected which are defective at different sites in the pathway of cell activation and secretion.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00042-12 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Chassy, Bruce N.	Research Chemist	LMI NIDR
Thompson, John	Expert	LMI NIDR
Lee, Yang J.	Visiting Fellow	LMI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Microbiology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.75	2.25	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The uptake and metabolism of carbohydrates by lactic acid becteria were studied. Sugar transport systems were analyzed by physiological, biochemical and molecular biological approaches. The structural gene for β-D-phosphogalactoside galactohydrolase (P-β-gal) determined by the 35 Kbp lactose plasmid isolated from Lactobacillus casei 64H (pLZ64) was cloned into pBR322 using Escherichia coli X1849 as the host organism. One recombinant plasmid (pLZ600) containing the 7.9Kbp Pst I β fragment of pLZ64 DNA, determined the enzyme. Minicell analysis of transformants conteining various pLZ600 subclones was performed. Results revealed the position and direction of transcription of the P-β-gal gene and a gene that encoded an unidentified 43 kdalton product. The gene encoding P-β-gal, but not the other gene, was transcribed from an L. casei-derived promoter. A physical map of restriction enzyme sites in pLZ64 was constructed. In other studies, it was demonstrated that 2-deoxy-D-glucose (2-DG) can inhibit growth of Streptococci without blocking metabolism via a futile cycle of 2-DG uptake, 2-DG 6-phosphate cleavage, and 2-DG expulsion that dissipates PEP.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00043-12 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Physiological and Genetic Studies on Pathogenic Oral Microorganisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Donkersloot, Jacob A.	Research Microbiologist	LMI NIDR

COOPERATING UNITS (If any)

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Microbiology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.00	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range objective of this project is to identify and characterize plasmids from oral streptococci, and to study their possible contribution to the physiology, ecology and pathogenicity of this group of organisms. Current studies have focused on a tetracycline (Tc) resistance determinant carried by an animal isolate of S. mutans. This determinant could transfer by conjugation to certain strains of S. faecalis and S. mutans. Whereas the transfer was accompanied by the appearance of a plasmid (pDJ2) in the S. faecalis transconjugants, no plasmid was evident in the original S. mutans host. Thus, the location of the Tc resistance determinant in this strain remained in question. pDJ2 was used as a probe to identify the Tc resistance locus in S. mutans 19S. Purified plasmid was labeled by nick-translation and hybridized to electrophoretically separated BindIII fragments of total cellular DNA. A unique 12 kilobasepair fragment was identified in all the Tc resistant strains. This result indicates that the Tc resistence determinant is encoded on a similarly sized plasmid in both the original S. mutans host and all the transconjugants. No evidence was found for a chromosomal Tc resistance determinant.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00045-11 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Macrophage, Keratinocyte, and Lymphocyte Mediators in Immunity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Oppenheim, Joost J.	Medical Officer	LMI NIDR
Luger, Thomas	Visiting Associate	LMI NIDR
Siraganian, Reuben P.	Medical Officer	LMI NIDR
Chou, Yuan K.	Visiting Fellow	LMI NIDR
Sztein, Marcelo	Visiting Fellow	LMI NIDR
Kasahara, Tadashi	Visiting Fellow	LMI NIDR
Scala, Giuseppe	Visiting Fellow	LMI NIDR
Charon, Jacques	Visiting Fellow	LMI NIDR
Nergenhagen, Stephan E.	Chief, LMI	LMI NIDR

COOPERATING UNITS (If any) B. Mathieson, NIAID; M. Mage, NCI; D. Sauder and S. Katz, NCI; J. A. Schmidt, NIAID; P. A. Murphy, Johns Hopkins Univ. Sch. Med., Baltimore, Md. G. Crabner, USC, San Francisco, Ca., J. Smolin and A. D. Steinberg, NIADDK

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.00	6.25	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Normal monocytes, keratinocytes and lymphocytes as well as cell lines when activated by antigenic or polyclonal stimulants produce a multiplicity of immunoregulatory mediators with potent biological effects on a wide variety of target cells at concentrations of 10^{-10} to 10^{-15}M. Activated macrophages produce interleukin 1 (IL 1) which enhances the proliferation of peanut nonagglutinating (PNA$^-$) thymocytes and induces them to produce the lymphokine IL 2, which in turn induces proliferation by PNA$^+$ thymocytes. The IL 1 has pleotrophic effects in that it stimulates hepatocytes to produce serum amyloid A (SAA), is a growth factor for fibroblasts and has endogenous pyrogen activity. Keratinocytes produce a mediator(s) that has the same biological and biochemical properties as IL 1. In addition, this epidermal cell derived thymocyte activating factor (ETAF) as well as IL 1 are chemotactic for neutrophils and monocytes. ETAF and IL 1 also activate neutrophils. IL 1 and or ETAF activities have been detected in the gingival exudate of normal subjects, and even more is detected in exudate obtained from sites of gingival inflammation. IL 1 and ETAF are important in initiating a cascade of interactions between cells and factors that modulate inflammatory responses.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00046-11 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chronic Inflammatory Disease and Lymphoid Cell Regulation of Connective Tissue Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Wahl, Sharon M.	Research Microbiologist	LMI NIDR
Wahl, Larry M.	Research Biologist	LMI NIDR
Gately, Celia	Staff Fellow	LMI NIDR

COOPERATING UNITS (If any) R. Wilder, NIAID

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Humoral Immunity Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations in this laboratory continue to focus on mechanisms of immune regulation of connective tissue metabolism. Lymphocytes and monocytes activated in vitro produce unique mediators which stimulate proliferation of fibroblasts. Furthermore, lymphocytes and monocytes isolated from inflamed synovial tissue or fluid of rheumatoid arthritis patients spontaneously release these factors emphasizing their potential role in the fibroplasia and fibrosis associated with chronic inflammatory lesions. Depletion of lymphocytes in patients with severe refractory rheumatoid arthritis by leukapheresis results in clinical improvement in some patients. This clinical responsiveness is associated with an enhancement of immune function and appears to be the result of depletion of a functionally abnormal subset of mononuclear cells. Furthermore, in an experimental rat model, the onset of streptococcal cell wall induced arthritis is the consequence of a deficient immune system in the genetically susceptible strains of rats. Thus, lymphocytes and monocytes appear to be responsible for the initiation and perpetuation of synovial inflammation in arthritis, and their products may provide the molecular link between the inflammatory response and the subsequent changes in connective tissue which accompany inflammation.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00061-09 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tumor Reactive Antibody with Chemotactic Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Sandberg, Ann L.	Research Biologist	LMI NIDR

COOPERATING UNITS (If any) R. Obrist, Dept. Internal Medicine, University Clinic, Basel, Switzerland

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Humoral Immunity Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50		.

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since macrophages are considered to be major effector cells in host defense against tumors, increasing their concentration at a tumor site should be of therapeutic value. Elevation of the numbers of macrophages in guinea pig hepatomas has been achieved by the in vivo administration of covalent conjugates of IgG antibodies reactive with tumor cell surface antigens and the chemotactic peptide, formylmethionylleucylphenylalanine (fMLP). These conjugates, which were chemotactic for guinea pig macrophages in vitro and bound to tumor cells but not to normal liver cells, fibroblasts or fibrosarcoma cells, significantly ($p < .005$) increased the numbers of macrophages in the tumors when administered either in a single dose or in five doses. Although five injections of unconjugated fMLP were nearly as effective as the conjugates, free fMLP did not enhance the numbers of macrophages in tumors when injected as a single dose. Unconjugated IgG was ineffective. The mean tumor weights were decreased in those groups of guinea pigs which received the conjugates but statistical significance was not achieved due to tumor weight variability in all groups.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZD1 DE-00131-08 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulatory Role of Thymus-Derived Lymphocytes on the In Vitro Antibody Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Farrar, John J.	Research Microbiologist	LMI NIDR
Benjamin, William R.	Postdoctoral Fellow	LMI NIDR

COOPERATING UNITS (if any)

M. Meltzer, NCI

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.50	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Macrophages, in conjunction with antigen or mitogen, stimulate T cells to produce a variety of immunoenhancing factors which serve to augment the immune responses of B cells, T cells, and macrophages. We have utilized phorbol myristic acetate-stimulatnd EL-4 mouse thymoma culture supernatants as the source of a variety of these mediators. A new factor, B cell growth factor (BCGF), distinct from a variety of other lymphokines has been described and shown to have a monomeric molecular weight of 12,000 and two isoelectric points of pH 6.4 and 7.4. The BCGF functions in synergy with interleukin 2 (IL 2) and T cell replacing factor to enhance the antigen-specific antibody response. Additionally, we have characterized two additional EL-4-derivnd soluble mediators which exhibit the capacity to activate macrophages to become tumoricidal. One of the factors co-purifies with gamma interferon (IFN-γ) whereas the second has a lower molecular weight and is clearly distinct from IFN-γ. Experiments have continued on the regulation of IFN-γ production by the development of a cloned T cell line which responds to PMA and IL 2 to produce very high titers of IFN-γ.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZD1 DE-00216-06 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunological Control of Connective Tissue Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Wahl, Larry M.	Research Biologist	LMI NIDR
Sandberg, Ann L.	Research Biologist	LMI NIDR
Wahl, Sharon N.	Research Microbiologist	LMI NIDR

COOPERATING UNITS (if any)

R. Wilder, NIAID
S. Weintraub, NIDR

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Humoral Immunity Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	2.25	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the role of the immune system in connective tissue metabolism. Studies examining the events involved in macrophage activation which lead to the production of collagenase have focused on the role of the ornithine decarboxylase (ODC) pathway. Our findings demonstrate that a significant inhibition of LPS-induced ODC occure when macrophages are exposed to indomethacin or difluoromethyl ornithine, a specific inhibitor of ODC. These agents also block collagenase production, implicating ODC in the activation by macrophages. Immune regulation of bone metabolism has been studied in osteopetrotic (op) rats and in rats in which rickets has been induced by a vitamin D deficient diet. Thymocytes from op rats were suppressed in their proliferative response when compared to normal littermates. Separation of the thymocytes into subpopulations by counterflow centrifugal elutriation demonstrated a subset of op cells which proliferated when stimulated. Studies of the immune function of normal and rachitic rats revealed that the lack of vitamin D results in a significant decrease in the proliferative capacity of thymocytes. Macrophage function is also affected as evidenced by a significant inhibition of in vivo and in vitro chemotaxis.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZD1 DE-00238-05 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Macrophage Functions in Immune Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Steeg, Patricia S.	Microbiologist	LMI NIDR
Oppenheim, Joost J.	Medical Director	LMI NIDR
Sztein, Marcelo	Visiting Fellow	LMI NIDR
Hakim, Francis	Guest Worker	LMI NIDR
Benjamin, William	Postdoctoral Fellow	LMI NIDR
Farrar, John J.	Research Microbiologist	LMI NIDR

COOPERATING UNITS (if any) H. M. Johnson, Univ. Texas at Galveston Med. Branch, Galveston, Texas; V. Kelley, Dept. of Med. and Immunogenetics, Brigham and Womens Hospital, Boston, Mass.; A. Steinberg, NIADDKD; R. Stiehm, Univ. of Calif., Div. of Immunol. and Allergy, Los Angels, Calif.; D. Mann, NCI.

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	3.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The initiation of antigen specific immune responses by macrophages requires cellular expression of Ia antigens. The in vitro regulation of macrophage Ia antigen expression was studied. We have found that a soluble mediator biochemically similar to immune interferon (IFN-γ) induces Ia antigen deficient (Ia^-) macrophages to express Ia antigens. Current research has focused on conditions that favor the Ia^+ state, which may be important in the immunosuppressed state. Bacterial endotoxin (LPS) inhibited IFN-γ induction of macrophage Ia antigen expression. This inhibitory effect was abrogated by indomethacin, a prostaglandin synthetase inhibitor, and mimicked by exogenous prostaglaadin E_2 and dibutyryl cAMP. The in vivo relevence of these in vitro regulatory pathways is being determined by correlation of macrophage Ia antigen expression and serum IFN-γ and PGE_2 levels. Finally the in vitro regulation of human monocyte Ia-like (D8) antigen expression has been studied. Peripheral blood monocytes, neonatal cord blood monocytes and HL60 cell line promonocytes expressed DR antigens in response to recombinant IFN-α.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DE-00242-05 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Role of Oxygen Radicals in Inflammation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Hoffeld, J. Terrell	Dental Officer	LMI NIDR
Charon, Jacques	Visiting Fellow	LMI NIDR
Oppenheim, Joost J.	Medical Director	LMI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Microbiology and Immunology

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our studies have further defined the role of oxygen radicals in models of immune inflammation in vitro and their potential bactericidal role in the gingival crevice, in vivo. Studies of the role of oxygen radicals in the suppression of lymphocyte responses by undegradable particles were concluded during this reporting period. These studies implicated oxidative damage as the mechanism of inhibition in an in vitro model of a low turnover granuloma. Preliminary studies of the thiol metabolism of murine spleen cells were initiated. These studies were predicated on the hypothesis that spleen cells protect themselves against oxidative damage by actively cleaving extracellular disulfide to free thiols. An assay for the measure of extracellular thiols in cultures was developed during this period. Finally studies of the crevicular neutrophils of normal, healthy adults were concluded. These studies showed that these cells have viability, oxidative metabolism, phagocytic capacity and chemotactic responsiveness equal to those of peripheral blood neutrophils. Thus crevicular neutrophils are fully functional as protective cells in the sulcus.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00254-05 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Microbial Antigens Associated with Specific Adherence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cisar, John O.	Research Microbiologist	LMI NIDR
Sandberg, Ann L.	Research Biologist	LMI NIDR
Mergenhagen, Stephan E.	Chief, LMI	LMI NIDR

COOPERATING UNITS (if any)
W. Clark, University of Florida, F. C. McIntire and A. E. Vetter, Univ. of Colorado Medical Canter

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Humoral Immunity Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.50	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Studies of the oral actinomycetes are continuing toward defining the adherence mechanisms of these bacteria to tissue surfaces. Investigations of the Actinomyces viscosus T14V cell surface have identified two distinct types of bacterial fimbriae (Ag1 and Ag2) that differ in their functional properties. The Ag2 fimbriae are the sites of a lactose-sensitive lectin activity that mediates coaggregation of actinomycete cells with certain plaque streptococci and the adherence of bacteria to mammalian cells. In contrast, those fimbriae designated as Ag1 or VA1 appear to play a critical role in bacterial adherence to saliva-treated beads of hydroxyapatite, an interaction that is unaffected by various sugars. The isolation and characterization of mutant bacterial strains lacking Ag1 fimbriae, Ag2 fimbriae or both components have provided additional support for the distinct functions of each structure. Morever, differences in the distribution of these fimbrial components on typical strains of A. viscosus and A. naeslundii appear to be correlated with certain well established differences in the adherence properties of these species. Thus, separate and specific structures on these bacteria seem to be involved in their adherence to different surfaces within the oral environment.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00273-04 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cell-Cell Interactions Between Oral Actinomycetes and Other Oral Bacteria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kolenbrander, Paul E.	Senior Staff Fellow	LMI NIDR
Cisar, John O.	Research Microbiologist	LMI NIDR

COOPERATING UNITS (if any)
University of Maryland, School of Dentistry

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Marylaod

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Cell-to-cell interactions (between oral bacteria) are most likely mediated by complementary surface components composed of a lectin on one cell type and a carbohydrate receptor on the other cell type. All of the coaggregatinns examined to date between oral actinomycetes (Actinomyces viscosus and A. naeslundii) and oral streptococci (Streptococcus sanguis. S. mitis, S. MG-intermedius, and S. mcebillourum) exhibit similar properties in that one cell type is resistant and the other is inactivated by heat or protease treatment. Many of these coaggregating pairs are inhibitnd by lactose, but others are unaffected by this sugar. Coaggregation-defective (COG^-) mutants that exhibit a single kind of surface structure that mediates coaggregation were used to probe some of these lactose-insensitive coaggregations. N-acetylneuraminic acid (sialic acid) inhibited a specific pair consisting of an A. naeslundii and a COG^- S. sanguis strain. The effect of human saliva on coaggregation properties of cells was also determined. Only minor differences in coaggregation properties of cells suspended under the two conditions were found.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00290-3 LMI

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Productinn of Hybridomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Siraganian, R.	Chief, Clinical Immunology	LMI NIDR
Fox, F.C.	Clinical Associate	LMI NIDR
Hook, W.A.	Research Microbiologist	LMI NIDR
Serfatti, D.	Sr. Asst. Dental Surgeon	LMI NIDR
Viswanathan, T.	Visiting Fellow	LMI NIDR
Basciano, L.	Microbiologist	LMI NIDR
Fischler, C.	Medical Technician Micro,	LMI NIDR
Serenstein, E.	Microbiologist	LMI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.50	3.00	2.50

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Hybridomas are being produced which secrete monoclonal antibodies of defined antigen sperificity and antibody subclass. Hybridomas have been producnd against Actinomyces viscosus, Cytophaga lymphokines, Fc$_\gamma$ receptor of mast cells and human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00316-02 LMI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biochemical Characterization of Biological Mediators in the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kraksuer, Teresa	Staff Fellow	LMI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Marylsnd

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.00	1.00	1.00

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Human monocytes are activated by endotoxin and other inflammatory microbial products to produce hormone-like factors such as interleukin 1 (IL 1). IL 1 enhances lymphocytes' mitogenic responses to lectins and thus appears to be a nonspecific lymphocyte activating signal that acts jointly with the antigen or polyclonal stimulant to induce T cell activation. IL 1 from human monocytes has been purified 20,000 fold by the sequential use of ultrogel AcA54 column, affinity chromatography and isoelectrofocusing. A human monocyte leukemia cell line (THP-1) was found to produce IL 1 upon stimulation and can potentially be used to produce more IL 1 for biochemical purification and molecular cloning of the IL 1 gene.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00317-02 LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
H_2O_2 Inducing Factor: Biochemical Characterization, Purification and Determination of its Role in Immune and Inflammatory Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cately, Celia L.	Staff Fellow	LMI NIDR
Oppenheim, Joost J.	Medical Director	LMI NIDR

COOPERATING UNITS (if any) T. Fleisher, WRAMC, Washington, D. C.; R. Fisher, NCI; F. Rollwagen, NIAID

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.00	1.00	1.00

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The ability of macrophages to release H_2O_2 has been correlated with their ability to kill bacteria and tumor cells in vitro. The mechanism by which the macrophage becomes activated to increase its production of H_2O_2, however, has not been clearly defined. A study has thus been made of a lymphokine, termed H_2O_2 inducing factor (H_2O_2 IF), which is produced by human T cells. H_2O_2 IF has been found to stimulate human monocyte-like cells to increase their production of H_2O_2, which is measured by means of a colorimetric microassay based on the peroxide-mediated oxidation of phenol red. Using this assay, the H_2O_2 IF has been determined to have a mw of 54,000 and an isoelectric point of 5.5. In addition, the factor has been determined to have a buoyant density of 1.307 g/ml, indicating that the molecule is a protein.

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00333-01 LMI

PERIOD COVERED
October 1, 1981 through September 31, 1982

TITLE OF PROJECT (80 characters or less)
Familial Aggregation of Oral Strains of Actinomyces Species in Humans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ssrfatti, David	Staff Fellow	LMI NIDR
Rams, Thomas	Staff Fellow	CIPCB NIDR

COOPERATING UNITS (if any)

Dr. Paul E. Keyes, International Dental Health Foundation, Reston, Va.

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
To further understand the transmission and colonization of periodontopathic microorganisms, the degree of intrafamily concordance of oral strains of Actinomyces viscosus and naeslundii is being determined by testing subgingival plaque samples of individual family members with specific monoclonal antibodies and immunofluorescence. The ability to "fingerprint" specific bacterial substrains found in dental plaque with monoclonal antibodies provides an epidemiological tool to study the transmissibility of microorganisms within families. A finding of significant familial clustering will imply that members of the same family tend to be more alike with respect to a particular strain of bacteria (Actinomyces) than individuals from different families, which is characteristics of many infectious and transmissible diseases.

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(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00341-01 LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Regulation of Sugar Transport and Metabolism in Lactic Acid Bacteria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Thompson, John	Expert	LMI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The growth of many strains of S. lactis (and L. casei) was inhibited by non-metabolizable glucose analogs including 2-deoxy-D-glucose (2DG) and 2-fluoro-2-deoxy-D-glucose. A novel futile-cycle has been discovered for 2DG-mediated inhibition of bacterial growth. In S. lactis the cycle involves three enzymatic steps: 1) accumulation of 2DG-6-phosphate via the phosphoenol-pyruvate (PEP) dependent glucose: phosphotransferase system (glucose-PTS), 2) intracellular hydrolysis of the phosphorylated derivative, and 3) efflux of free 2DG. This futile cycle promotes the dissipation of glycolytic PEP and the non-metabolizable analog functions as an uncoupler by dissociating energy generation from bacterial growth. The immunity to 2DG exhibited by some S. lactis strains is achieved by fine, and coarse regulation of the activity of the glucose-PTS. These short, and long-term responses reduce the extent of futile re-cycling and energy is conserved for growth. In separate projects it has been shown: A) that glucose-PTS defective mutants may be isolated by positive selection for resistance to 2DG, B) that separate galactose-, and lactose; PTS systems are present in L. casei and C) cells of S. lactis contain an intracellular hexose-6-phosphate phosphohydrolase and this enzyme has been purified and characterized.

PHS-6040
(Rev. 2-81)

LABORATORY OF BIOLOGICAL STRUCTURE

The research efforts of the Laboratory of Biological Structure have continued to provide significant new information on the chemistry, structure and function of the tissues related to the oral cavity. While our efforts remain focused on understanding normal tissue components and their physiological roles, important new steps have been taken in elucidating the molecular pathology of certain skeletal diseases. The progress of the various LBS research groups is summarized below.

SKELETAL MATRIX BIOCHEMISTRY SECTION

Continued progress has been made in the elucidation of the structural and chemical characteristics of bone matrix proteins. A major bone protein of approximately 70,000 daltons has been identified as the bone sialoprotein. Initial work in the early 1960's described a bone sialoprotein of 23,000 daltons, but studies in our laboratory failed to identify a similar protein in this size range. It now appears that the 23,000 dalton sialoprotein is a proteolytic fragment of the 70,000 dalton molecule. Antibodies raised to the small (70-120,000 dalton) bone proteoglycan have been shown to cross react only with dentin extracts, and minimally with purified scleral proteoglycans, emphasizing the relatively unique nature of this molecule. Immunocytochemical studies localized the proteoglycan to osteoblastic and osteoprogenitor cells, suggesting that this molecule may be the first bone specific gene product produced during osteogenesis. A second proteoglycan associated with the loose connective tissue between trabeculae of developing intramembranous bone has now been identified. It is considerably larger (750,000-1,200,000 daltons) than the bone specific proteoglycan, and its core protein is chemically and immunologically related to the cartilage proteoglycan.

Important data on the potential biological function of these bone matrix proteins have been obtained. A protein of about 62,000 daltons has been found to be chemotactic for osteoblasts *in vitro*, and may serve as a local recruitment factor for preosteoblastic cells. The 24,000 dalton phosphoprotein described last year appears to have some relationship to bone remodeling. Increased levels of this phosphoprotein were associated with spongy bone spicules undergoing active remodeling, and it inhibited resorption of rat bone rudiments *in vitro*. Studies of developing and aging bone revealed relatively constant levels of the bone specific proteins, with an increase in their apparent breakdown products occurring in aging bone. In contrast, considerable variation in the levels of α_2-HS-glycoprotein and osteocalcin, which could not be related to bone formation or remodeling, were found. Finally, levels of osteonectin were measured in animal models of two bone diseases, rickets and osteopetrosis. Osteonectin was markedly reduced with the impaired mineralization of rickets, while greatly increased levels occurred in the hypermineralized bone of osteopetrotic animals. These findings are a significant first step towards the elucidation of the molecular basis of bone pathology.

MINERAL CHEMISTRY AND STRUCTURE SECTION

Studies conducted during the past year have focused on two main areas: cell associated mineralization; and the effects of proteins on hydroxyapatite crystal growth. Synthetic studies of ionophore mediated calcium transport showed that the initial crystalline phase formed had an apatite-octacalcium phosphate interlayered structure. Additionally, formation and growth of this solid phase was closely related to the cation exchange reactions occurring at the aqueous/organic solvent interface. Continued studies employing modifications of this system are expected to provide important data on matrix vesicle calcification processes. Initial work on characterizing intramitochondrial precipitates utilized synthetic models to provide information on butyrate-induced inorganic ion changes in hepatic mitochoneria. The precipitation reaction appears to be complex, occurring in more than one mitochondrial compartment, or involving enzymatic hydrolysis of pyrophosphate or binding to organic substances. Understanding of the reactions involved in mitochondrial electrolyte metabolism may shed light on the role of mitochondria as an intracellular mineral source in calcifying tissues.

Initial work on the role of enamel proteins in apatite crystal growth has shown that the presence of enamelins, which remains associated with enamel crystals after guanidine extraction, has no effect on seeded crystal growth in metastable solutions. Electron microscopic analysis showed that new crystal growth occurred at the ends of the seed crystals, and that the appearance of the new growth was dependent on the saturation of the solution with respect to octacalcium phosphate. New studies begun on physiologically signifcant inhibitors of calcification have demonstrated the presence of macromolecular inhibitors in rat and human plasma. Inhibitor activity in human plasma was associated with molecules of approximately 80,000 and 50,000 daltons. An attempt to identify these inhibitors is in progress. These studies should provide important information on the regulatory role of proteins and other macromolecules in normal and pathological calcification processes.

BONE CELL BIOLOGY SECTION

The matrix-induced endochondral bone formation system continues to be a useful model for the study of the differentiation, growth and mineralization of cartilage and bone. Work during the past year has focused on the role of vitamins A and D and insulin. Vitamin A caused a marked reduction in mesenchymal cell proliferation, chondrogenesis, and mineral incorporation into bone. Cartilage proteoglycan synthesis was also altered, resulting in the production of a smaller molecular weight proteoglycan. The effects of vitamin D were assessed by treatment of D-deficient animals with the various vitamin B metabolites. $24,25(OH)_2D_3$ stimulated chondrogenesis and bone formation, while $1,25(OH)_2D_3$ stimulated increased bone remodeling and resorption. Bone marrow formation was also shown to be dependent on the vitamin D status of the animals. In D deficiency there was a reduced number of spleen colonoy forming units and an increase in the rate of cell cycling. These effects appeared to be independent of plasma calcium levels and suggest a specific role for vitamin D in providing a microenvironment conducive to marrow formation. Previous work had demonstrated the profound effects of insulin on cartilage and bone formation. It has now been shown that the impaired mesenchymal cell proliferation is due to direct effects of insulin on the cells, rather than through systemic effects. Cell attachment to the implanted matrix may be an important factor in regulating proliferation. Local injections of insulin or fibronectin corrected the reduced cell attachment seen in diabetic animals. Thus, one mechanism of insulin action on bone differentiation may be the regulation of fibronectin synthesis.

As reported last year, a major effort is being directed toward elucidation of the biochemical events involved in endochondral bone formation. Chemotactic and mitogenic factors have now been identified in guanidine extracts of the demineralized bone matrix. The mitogenic factors appear to be specific for fibroblasts, and are present in the extract fractions shown to have osteoinductive properties. These growth factors probably play a significant role in the local regulation of bone differentiaion and growth. Through a systematic survey of the glycoproteins synthesized during the development of endochondral bone, additional molecules with specific functions in bone formation may be identified. For example, on days 9-11 during the onset of mineralization, the transient synthesis of a 32,000 dalton mannose-containing glycoprotein has been demonstrated. This glycoprotein appears to be synthesized by hypertrophic chondrocytes, and may be important for initiation of mineralization, vascular invasion or recruitment of bone cells.

EXPERIMENTAL MORPHOLOGY SECTION

Saliva plays a major role in regulating the oral environment. Studies conducted during the past year have provided new information on cellular mechanisms involved in the production and secretion of salivary constituents. Electron microscopic studies employing cytochemical tracers of various molecular weights have shown that secretory stimulation with β-adrenergic agonists increases the permeability of the "tight" junctions which join adjacent cells and form as barrier between the interstitial space of the gland and the lumen. Molecules up to $\sim$35,000 daltons are able to penetrate the junctions following isoproterenol stimulation. These findings suggest that certain components of the extracellular fluid may gain access to the saliva via a paracellular route. Intracellular alterations induced by β-adrenergic stimulation are mediated by cyclic-AMP-dependent protein kinases. Stimulation-induced redistribution of the protein kinase isozymes appears to be one mechanism for regulating their activity. Using a photoaffinity labeling procedure, protein kinase subunits which bind cyclic AMP (regulatory subunits) have been identified in rat and human parotid saliva. The function of these intracellular proteins in saliva is unknown. Their presence may be related to the attendent stimulation-induced compartmental redistribution, or simply a consequence of their close association with secretory granule proteins.

Stimulation of secretory activity by exocrine cells also results in increased endocytic activity at the cell surface. Part of this endocytic activity is related to retrieval of secretory granule membranes added to the luminal surface during exocytosis. Receptor-mediated endocytosis apparently occurs at the lateral and basal cell surfaces, and results in the sequestration of exogenous tracers in a system of basal tubular lysosomes. These lysosomes have been identified in a number of eifferent exocrine cells and hav unique cytochemical properties. The eventual sorting out of the various intracellular pathways of endocytosed membrane is being approached through the use of monoclonal antibodies directed toward cell surface components.

LABORATORY OF BIOLOGICAL STRUCTURE

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00012-20 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Infrared and Raman Spectroscopic Studies of Teeth and Bones and Related Synthetic Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Fowler, B.O.	Research Chemist	LBS NIDR
Lenk, E.V.	Expert/Consultant	LBS NIDR

COOPERATING UNITS (If any)

1) Dr. S. Kdroda, Tokyo and Dental University, Japan

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Mineral Chemistry and Structure Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.36	1.05	0.31

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (e.g., hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependency and polarization) are then utilized to establish compositional and structural details of the apatites in question which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00028-15 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ultrastructure and Cytochemistry of Secretory Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Hand, A.R.	Chief, LBS	LBS NIDR
Oliver, C.	Research Biologist	LBS NIDR
Lenk, E.V.	Expert/Consultant	LBS NIDR
Quarnstrom, E.E.	Visiting Fellow	LBS NIDR
Mednieks, M.I.	Senior Staff Fellow	LBS NIDR
Mezariegos, N.R.	Visiting Fellow	LBS NIDR
Wolf, E.D.	Dental Director	LBS NIDR
Whitson, S.W.	Guest Researcher	LBS NIDR

COOPERATING UNITS (If any)

1. Dr. Lois Tice, LEP, NIADDR;

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Experimental Morphology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.72	2.93	1.79

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Basic mechanisms of the secretory process are studied in cells of the rat pancreas, salivary and lacrimal glands. Techniques utilized include light and electron microscopy, cytochemistry, radioautography, and basic biochemical procedures. Major areas of investigation are: (1) the structure and function of the Golgi apparatus and GERL; (2) experimental pathology and lysosome function in salivary glands; (3) structure and permeability properties of junctional complexes in the rat parotid gland; and (4) the effects of sialographic procedures on the structure and function of the rat submandibular gland.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00074-10 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Bone and Tooth Matrix Biochemistry and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Termine, J.O.	Res. Chem.	LBS NIDR	Eanes, E.D.	Res. Chem.	LBS NIDR
Shimokawa, H.	V. Assoc.	LBS NIDR	Doi, Y.	V. Fellow	LBS NIDR
Fisher, L.W.	Guest Worker	LBS NIDR			
Pincham, A.G.	V. Scientist	LBS NIDR			
Kleinman, H.K.	Res. Chem.	LDBA NIDR			
Hassell, J.R.	Res. Biol.	LDBA NIDR			
Somerman, N.J.	Staff Fellow	CIPC NIDR			
Drum, N.A.	Clin. Staff Dent.	CIPC NIDR			
Hascall, V.C.	Res. Chem.	LB NIDR			
Yanagishita, N.	Expert	LB NIDR			
Wahl, L.W.	Res. Biol.	LMI NIDR			
Reddi, A.H.	Res. Biol.	LBS NIDR			
Wientroub, S.	V. Assoc.	LBS NIDR			

COOPERATING UNITS (If any)

1) Dr. S.W. Whitson, SIU, School of Dentistry
2) Dr. L. Raisz, University of Connecticut Medical School

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Skeletal Matrix Biochemistry Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.89	3.14	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biochemical and metabolic properties of developing skeletal and dental tissues are being studied by several techniques. Bone, dentin, and enamel matrix proteins are investigated as to their structural and functional roles in skeletal tissue processes. Emphasis is placed on phosphoprotein and glycoprotein biochemistry in these hard tissue matrix studies. Bone and tooth formation and mineralization are studied using in vitro methodology.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00088-09 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemical, Structural, and Morphological Studies on Calcium Phosphates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Eanes, E.D.	Research Chemist	LBS NIDR
Doi, Y.	Visiting Fellow	LBS NIDR
Termine, J.O.	Research Chemist	LBS NIDR

COOPERATING UNITS (If any)

1) Dr. R.L. Veech, NIAAA, ADAMHA
2) Dr. Jonathan L. Costa, NIMH, ADAMHA

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Mineral Chemistry & Structure Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.30	1.40	0.90

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The properties of calcium phosphate salts of biological interest are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, B-E-T surface area methods, chromatographic and standard analytical chemistry procedures. Topics under current investigation include (1) the preperation and characterization of synthetic analogues to intracellular mineral deposits such as occur in mitochondria and in subcellular storage orgnelles, (2) the formation and properties of precipitates induced in phosphate solutions by the ionophoric translocation of Ca ions across lipophilic solvent barriers, and (3) the modulation by enamel proteins of crystal growth processes in supersaturated solutions seeded with apatite.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00162-06 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Kinetic and Thermodynamic Characterization of Calcium Phosphate Precipitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Meyer, J.L.	Research Chemist	LBS NIDR

COOPERATING UNITS (if any)

1) Dr. B. Fleisch, Pathophysiology Institute, University of Bern, Bern, Switzerland.

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Mineral Chemistry & Structure

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.07	1.0	.07

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to determine the thermodynemic and kinetic factors which regulate the nucleation, crystal growth and maturation of calcium phosphate crystals. This is accomplished by estimating free ionic activities in solution for all species involved in the crystallization process and relating these terms to the observed precipitation steps. A further correlation is then made between the composition of the solution and the properties of the solid calcium phosphate phase in equilibrium with it. The effect of crystallization inhibitors on the precipitation of calcium phosphates is also being studied in order to elucidate their mode of action at crystal surfaces. Emphasis is placed upon inhibitors which occur naturally in physiological systems or which are common therapeutic agents.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00199-06 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

In Vitro Studies of Secretory Cell Structure and Function.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dliver, C.	Research Biologist	LBS NIDR
Hand, A.R.	Chief, LBS	LBS NIDR
Lenk, E.V.	Expert/Consultant	LBS NIDR
Yuasa, Y.	Guest Worker	LBS NIDR
Siraganian, R.	Chief, CI	LMI NIDR
Robbins, A.	Sr. Staff Fellow	GB NIADDK

COOPERATING UNITS (if any)

NCI, FOB.

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Experimental Morphology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.38	1.85	1.53

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Secretory and endocytic processes in several cell types are currently under investigation. Cell dissociation and short term culture (up to 1 month) methods have been established for rat exorbital lacrimal, parotid and pancreatic acinar cells. These cultures are being used to study various aspects of the secretory process. Emphasis is being placed on morphological, cytochemical and biochemical characterization of the cultured cells. Uptake and fate of both soluble phase and membrane bound markers by exocrine acinar cells is also being examined in vivo and in vitro.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00204-05 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Extracellular Matrix and Bone Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Reddi, A.H.	Research Biologist	LBS, NIDR
Kuberasampath, T.K.	Visiting Fellow	LBS, NIDR
Nientroub, S.	Visiting Associate	LBS, NIDR
Wloderski, K.	Visiting Scientist	LBS, NIDR
Tian, H.Y.	Guest Worker	LBS, NIDR
DeSimone, D.P.	Biologist	LBS, NIDR
Somerman, M.J.	Staff Fellow	CIPC, NIDR
Termine, J.D.	Research Chemist	LBS, NIDR
Hand, A.R.	Chief, LBS	LBS, NIDR

COOPERATING UNITS (if any)

1)Dr. Robin Poole, Shriners Hospital, Montreal, Quebec, Canada; 2) Dr. Lawrence Rosenberg, Montefiore Hospital, Broox, NY; 3) Dr. H. Hagan, AFFRI.

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Bone Cell Biology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.25	4.21	2.04

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to investigate matrix-cell interactions in bone. We employ an experimental model of matrix-induced cartilage and bone differentiation and mineralization. Projects currently under investigation are: 1) the mechanism of action of matrix components in bone induction; 2) influence of local insulin on bone formation; 3) influence of diabetes on fibronectin and matrix-cell interactions; 4) role of vitamin D metabolites in bone formation; 5) experimental rickets and bone marrow function; 6) effect of vitamin A on bone differentiation; and 7) proteoglycans and mineralization of bone.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00217-04 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982.

TITLE OF PROJECT (80 characters or less)

Salivary Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Wolf, R.D.	Dental Director	LBS NIDR
Nubbard, V.S.	Physician	PM NIAMDD
Papadopoulos, N.	Biochemist	CC CP
Kingman, A.	Statistician	CPR NIDR

COOPERATING UNITS (if any)

1) Georgetown University School of Dentistry, Washington, D.C.
2) S.D. James, Ph.D. - USN Surface Weapons CTR

LAB/BRANCH: Laboratory of Biological Structure

SECTION: Experimental Morphology Section

INSTITUTE AND LOCATION: NIDR NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.46	0.90	0.56

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with mechanisms of production and control of extrinsic (i.e., saliva) and intrinsic (e.g., serum salivary isoamylase) salivary gland products. Human and animal (primarily parotid) saliva chemical constituents and mechanisms are evaluated as related to health, disease and physiological state. Parotid salivary flow rate, protein content and enzymes (particularly lysozyme and amylase) are evaluated in normals and selected disorders. The intrinsic secretion of salivary isoamylase in serum of cystic fibrosis of the pancreas is being studied. Diagnostic application and an understanding of the mechanisms of hyperamylasemia are being sought.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 OE 00285-03 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Protein Secretion in Salivary Glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mednieks, M.I.	Senior Staff Fellow	LBS NIDB
Hand, A.R.	Chief, LBS	LBS NIDB
Wolf, R.O.	Dental Director	LBS NIDR

COOPERATING UNITS (if any)
1) De Wys, W., Div. Canc. Treatment, Clin. Inv. Branch, NCI; 2) Jungmann, R.A., Northwestern University; 3) Dowd, P., Creighton University.

LAB/BRANCH
Laboratory of Biological Structure

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.06	1.14	0.92

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Molecular mechanisms of action are studied in parotid gland acinar cells to determine regulatory events associated with protein exocytosis. In addition to standard biochemical, immunological and morphological methods recently developed experimental techniques such as photoaffinity labelling (8-azido cyclic [32P]-AMP), enzyme linked immunosorbent antibody technique (ELISA) and microscopic examination of subcellular fractions at the LM and EM level are part of the experimental design. Cellular responses to receptor interactions of parotid cells with B-agonists have been studied using measurements of cyclic AMP-dependent protein phosphorylation as an index. The activity is both extra-nuclear as well as associated with chromatin-bound nonhistone nuclear proteins. Redistribution of protein kinase isozymes occurs after stimulation with isoproterenol. Additional cyclic AMP-binding proteins (cAMP'PK regulatory subunits have been identified in human and rat saliva. Transcribable (poly A) mRNA has been isolated from rat parotid tissue for determining stimulation-induced gene regulation of secretory protein synthesis using specific antibody to a-amylase as the immunological reagent.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00335-01 LBS

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Control of Mineralization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Zaki, A. Moneim	IPA	LBS NIDR
Hand, A.R.	Chief, LBS	LBS NIDR

COOPERATING UNITS (if any)
1) Dept. of Histology, School of Dentistry, University of Illinois, Chicago, IL
2) BBIB, DRS, NIH

LAB/BRANCH
Laboratory of Biological Structure

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.13	1.02	0.11

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our long term objective is to contribute to a better understanding of the cellular role in mineralization. The distribution and movements of calcium in and between cells associated with mineralizing enamel and dentin currently remain elusive. One approach to this problem is to investigate membrane-associated enzymes which may influence the movements of calcium ions during mineralization. Certain phosphatases present in secretory and non-secretory ameloblasts and odontoblasts of frogs teeth are bein localized by light and electron microscopic cytochemistry and assayed biochemically during different stages of amelogenesis and dentinogenesis. A second approach to this problem is to directly or indirectly localize calcium in the tissues. Autoradiography of Ca-45, calcium precipitation with potassium pyroantimonate, x-ray dispersive analysis, and electron energy loss spectroscopy have been employed to study the distribution of calcium in incisors of normal and experimentally treated rats.

PHS-6040 (Rev. 2-81)

LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES

Research activities in the Laboratory of Developmental Biology and Anomalies are concentrated on normal and abnormal development, on wound healing and on various acquired and inherited disorders. We use rather diverse approaches in these research areas, including genetics, molecular biology, cell biology, biochemistry, and animal experimentation. During the last year, the number of laboratory personnel has remained relatively stable.

RESEARCH ACCOMPLISHMENTS

STRUCTURE AND FUNCTION OF BASEMENT MEMBRANES

In 1977 we reported that a transplantable murine tumor, the EHS tumor, produced quantities of basement membrane. At that time, basement membranes were not well described, since in normal tissue the basement membranes are minute, metabolically inert and insoluble. Basement membranes are of considerable interest since they play a central role in development by forming the scaffolding along which tissues are organized. Further since they regulate the passage of macromolecules they are important to the filtration function of the kidney and blood vessels. In addition, they are involved in a variety of disorders including diabetes, immunologic diseases (such as Bullous pemphigoid, Chagas, etc.) and invasive cancers.

Our approach was to isolate proteins from the tumor, use them to prepare antibodies and use the antibodies in immunofluorescence to determine if similar proteins are present in authentic basement membranes. Using this approach, we discovered laminin, the epithelial attachment protein and a heparan sulfate proteoglycan, unique to basement membranes. The tumor proved to be a good source of type IV collagen. Using the antibodies to these molecules, we have found that type IV collagen, laminin and heparan sulfate proteoglycan are present together in basement membranes where they form an integrated structure. All basement membranes contain these three components. Basement membranes are produced very early in the formation of a tissue and are then maintained throughout its development. We are studying the structure of these components and using them to culture cells and to reconstitute the basement membrane *in vitro*.

We are studying the organization of these materials in basement membrane and their interactions with one another. Type IV collagen is stablized in the basement membrane by disulfide bonds and by lysine-derived crosslinks. Our studies also establish that the type IV collagen molecule in the matrix is the same size as the molecule made by the cell. Other collagen types, in contrast, arise in precursor forms which are enzymatically cleaved to produce shortened molecules for the matrix. Laminin is bound by noncovalent bonds to type IV collagen and the heparan sulfate proteoglycan is bound to laminin. In reconstitution experiments, we find that neither type IV collagen nor laminin precipitates under physiological conditions. However, when mixed together an aggregate structure is formed which precipitates. The heparan sulfate proteoglycan binds to the laminin-type IV collagen complex.

SYNTHESIS OF BASEMENT MEMBRANE

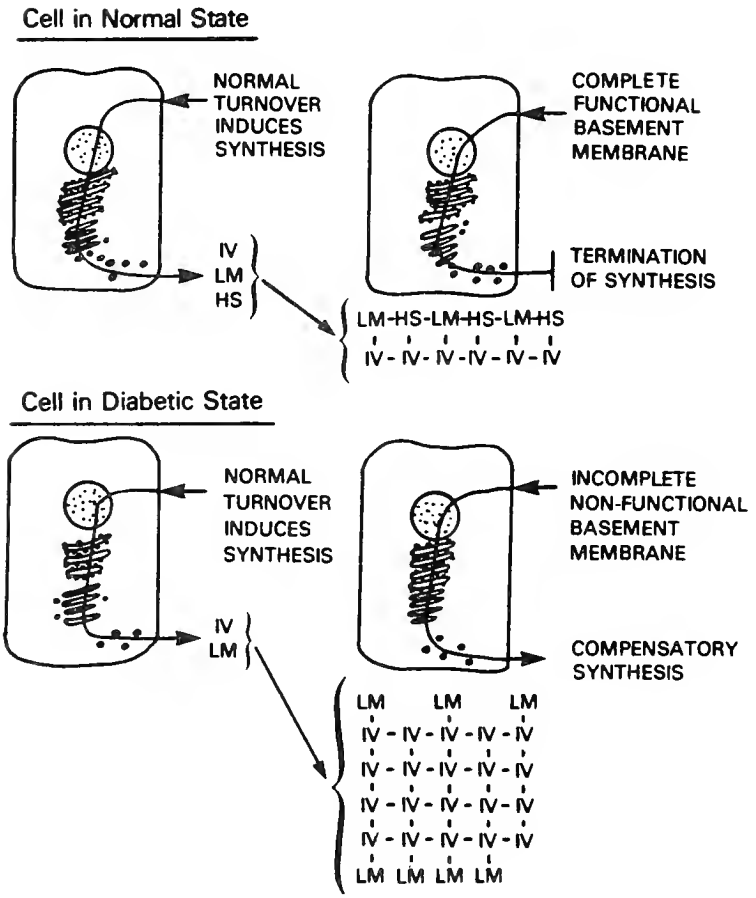

Figure I legend. Turnover of basement membrane initiates the synthesis of type IV collagen (IV), laminin (LM) and heparan sulfate proteoglycan (HS). These proteins are assembled into the functional basement membrane. The lack of heparan sulfate proteoglycan in the diabetic state leads to a compensatory synthesis of laminin and type IV collagen.

DISORDERS INVOLVING BASEMENT MEMBRANE

Diabetes

Indiabetes, the basement membranes in capillaries, glomeruli, around nerves etc. become grossly thickened. Although thicker, the basement membranes are more porous than normal. This permeability change alters normal tissue metabolism leading to severe periodontal disease, blood vessel disease, blindness, kidney failure, neuropathies and a shortened life span.

We have studied the components of basement membrane produced by the EHS tumor grown in normal and in diabetic mice. These studies show normal or increased amount of protein and laminin in diabetic tissue but very low levels of the basement membrane specific (heparan sulfate) proteoglycan. In fact, there appears to be an inverse relation between serum glucose and proteoglycan synthesis. Reduced synthesis is observed at levels of glucose only slightly above normal levels. Administration of insulin restores the synthesis of proteoglycan to normal. We have used these observations to explain the increase in basement membrane in diabetes. We propose (Figure 1) that the normal process of turnover induces the synthesis of basement membrane. Synthesis terminates when the basement membrane is complete and functional. In the diabetic tissue which lacks the proteoglycan, there is a continued compensatory synthesis of type IV collagen and laminin but the basement membrane, lacking proteoglycan is not functional.

EFFECTS OF ATTACHMENT PROTEINS ON CULTURED CELLS

Fibronectin, laminin and chondronectin mediate the cell-substratum attachment of fibroblasts, epithelial cells and chondrocytes respectively. Now we have investigated the effect of these attachment proteins on certain other cell types. Laminin is found to stimulate the rate and density of outgrowth of neurites from fetal human sensory ganglia. Schwann cells are found to make laminin and utilize it for attachment. Fibronectin exerts a strong mitogenic effect on Schwann cells although they do not utilize fibronectin for attachment or synthesize it. These factors may be useful in promoting the growth and differentiation of injured nerve tissue.

Related studies on the effects of attachment proteins have been carried out with fibroblasts and epithelial cells. Fibronectin stimulates the attachment of fibroblasts and therefore their growth in culture. Laminin, in contrast, inhibits the growth of fibroblasts but stimulates the attachment and the growth of epithelial cells. We find that there are separate binding sites for fibronectin and for laminin on type IV collagen. Thus, either fibroblasts or epithelial cells can attach to this collagen. However, when laminin binds to type IV collagen, it blocks the fibronectin binding site and thus prevents fibroblasts from attaching. Similar mechanisms may operate *in vivo* to exclude fibroblastic cells from epithelial and endothelial structures.

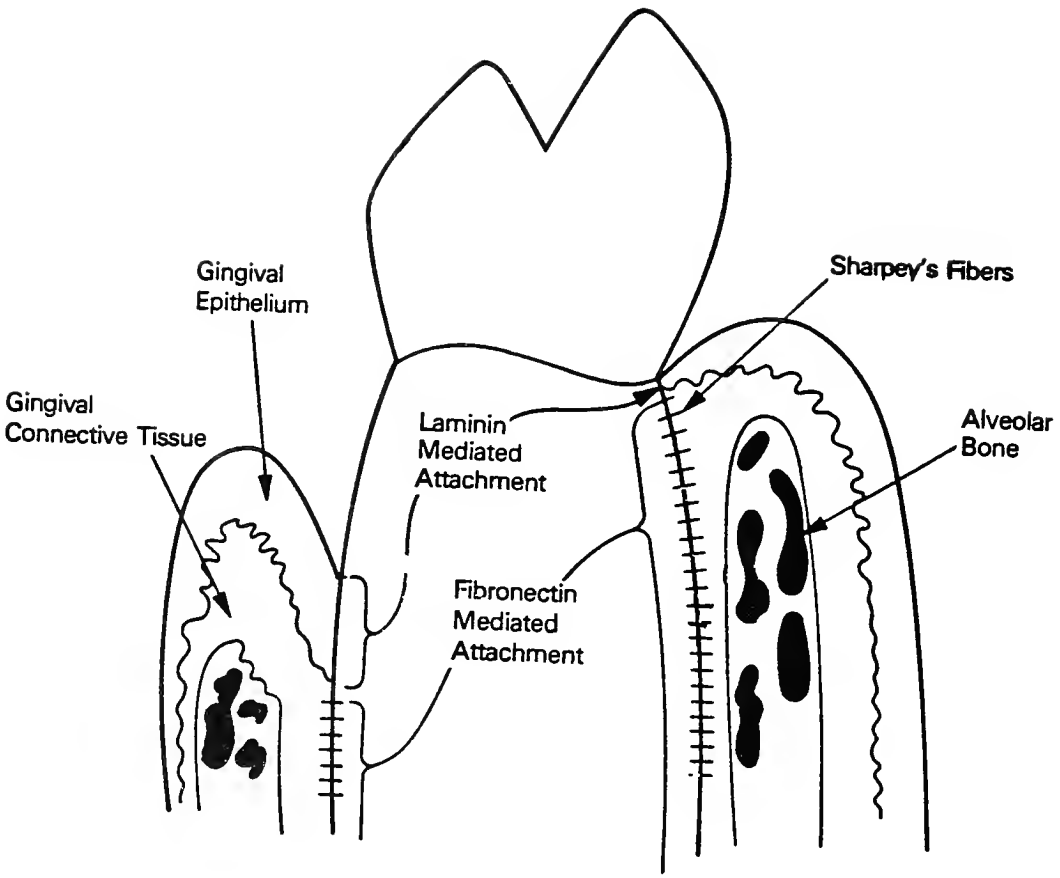

Figure 2 legend. Location of attachment factors along the tooth-gingival interface. The widened epithelial interface on the left side of the tooth involved in periodontal disease shows enlarged area of gingival epithelial cells in contact with the root surface over that seen on the uninvolved (right) side of the tooth which is adherent to the root surface fibronectin.

ATTACHMENT OF CELLS TO TOOTH SURFACES

Progress has been made in defining the factors that destroy the attachment of the periodontium to the teeth in periodontal disease. However, it is not clear why little or no restoration of normal attachment occurs when the disease process is arrested. We have studied the attachment of epithelial cells and fibroblasts to tooth surfaces *in vitro*. These studies show that cells will not attach to tooth surfaces exposed by periodontal disease unless the surfaces are cleaned. The material removed from the tooth in the cleaning process is cytotoxic and may well be endotoxin as reported by others. In addition, cells attach much better to root than to crown surfaces suggesting that specific mechanisms are involved. Epithelial cells attach and grow on these cleaned surfaces better than fibroblasts. However, if the tooth surface is exposed briefly to citrate (which causes some surface demineralization) and fibronectin is added, fibroblasts grow much better than epithelial cells. This procedure is relatively simple and could be adapted to a clinical situation to test the importance of restoring the interaction of gingival mesenchyme with the tooth surface. These and recent immunohistological studies on the localization of fibronectin and laminin at the soft tissue-tooth surface junction (diagrammed in Figure 2) show that the detached gingiva is more dependent on the weaker laminin mediated cell adhesion than is the healthy tissue which is held to the root surface primarily by fibronectin.

TUMOR METASTASES

In previous work, we found that metastatic tumor cells attached preferentially to basement membrane collagen over other collagen types and that these cells used laminin for attachment. The biological significance of these results was confirmed when tumor cells mixed with antibodies to laminin were injected into mice and found to be unable to metastasize. These results indicate that the ability of tumor cells to bind to basement membranes (through laminin) is essential to their spreading into tissues. Now we have found that there are specific cell surface receptors for laminin. The metastatic cells have many more receptors than tumorigenic, nonmetastatic cells. It is possible that one may assess the metastatic potential of tumor cells by measuring laminin receptor levels.

TRYPANOSOMAL INFECTIONS AND AUTOIMMUNITY TO BASEMENT MEMBRANES

We have found that humans and monkeys infected with Trypanosoma cruzi develop high titers of antibodies reacting with basement membranes. These antibodies are shown to be directed to laminin. Such antibodies are not found in other parasitic disorders with the exception of African trypanosomiasis where high levels of antilaminin are also produced. The antilaminin antibodies produced after trypanosomal infection are able to block the attachment of endothelial cells and in this way damage blood vessels. This could account for some of the degenerative changes observed in patients infected with these organisms.

The cause of this antibody induction is not known. Presumeably antibody is produced by the host to various proteins present on the parasite. We have recently observed that the antilaminin antibodies react strongly with the cell surface of the infectious form of the parasite suggesting that there is a surface protein immunologically related to laminin. Presumeably such an attachment protein would aid the parasite in binding to basement membranes during its penetration into tissue. However, the antibody produced the cell surface of the infectious form of the parasite would crossreact with laminin and of the host tissues and cause an immunological disorder. It is possible that one may assess the degree of infection by the antilaminin levels and eventually develop antisera specific to the parasite.

NORMAL AND ABNORMAL WOULD HEALING - THE CHEMOTAXIS CASCADE

Following trauma, cells enter the wound in a staged and ordered fashion. Platelets are the first to accumulate at the wound site. Others arrive in succession in the following order: polymorphonuclear leukocytes, macrophages, fibroblasts and then endothelial cells which form capillaries. Chemotactic factors specific for each cell type are believed to attract the cells.

We have identified various chemotactic factors that are specific for each cell type involved in the wound response. Products produced during the clotting reaction and bacterial metabolites attract polymorphonuclear neutrophils and macrophages. Platelets release a polypeptide hormone with mitogenic and chemotactic activity, the platelet-derived growth factor (PDGF). We find that PDGF is a potent chemoattractant for fibroblasts and for smooth muscle cells, but not for other cell types. In addition, we find that fibroblastic cells produce attractants for endothelial cells. These studies suggest that each wave of cells entering the wound produces the chemoattractant bringing in the next cell type and that a series of chemoattractants control the order and the time in which the cells appear.

A CHEMOATTRACTANT FROM BONE

Others in NIDR (Termine and Reddi, LBS) are isolating proteins from demineralized bone matrix. Bone matrix when implanted beneath the skin induces mesenchymal cells to migrate to the site where they differentiate into chondrocytes and produce cartilage.

This cartilage is soon replaced by bone. We find that extracts of the bone matrix contain a potent chemoattractant for osteoblasts. Activity resides in a single protein (M_r=65,000) distinct from other known bone proteins, including osteonectin or osteocalcin. This protein could serve an essential role in fracture healing by attracting the cells that repair bone.

ANTICHEMOTAXIS FACTORS FROM TUMORS

Certain tumors appear to impair the bodies ability to fight infectins. In studying a murine carcinoma, we found that this tumor secreted substance that prevented phagocyte chemotaxis. These materials have been isolated and found to comprise 3 low molecular weight peptides. Their structures and mechanism of action are under study.

FIBROSIS: ABERRANT WOUND HEALING

Current concepts suggest that fibrosis results from repeated or sustained tissue damage which elicits an excessive or inappropriate deposition of collagen. Fibrotic tissue is also deposited around implants and around many tumors. We are studying the mechanisms which underlie and induce the fibrotic process. Several different systems producing fibrosis are under study. Fibrous capsules around tumors, granulomas around shistosomes, and cirrhotic livers have been found to contain a significant proportion of type V and type III collagen. In contrast, healing skin wounds produce little or no type V collagen. These proportions of type V and type III collagen are characteristic of the spectrum of proteins made by smooth muscle cells and quite different from those made by fibroblasts. These studies suggest that smooth muscle cells, rather than fibroblasts or parenchymal cells, may be responsible for the deposition of collagen in fibrotic conditions.

It is possible that the smooth muscle cells are brought to the site of fibrosis by chemoattractants. We have found that certain human tumor cells which induce a strong desmoplastic response secrete a potent chemoattractant for smooth muscle cells and fibroblasts. Similarly, liver cells exposed to chemicals that induce fibrosis of the liver produce chemoattractants for smooth muscle cells and fibroblasts. The Kupffer cells in the liver appear to be the source of the chemoattractants.

FACTORS STIMULATING REPAIR

We have found that we can enhance the rate of wound healing. In these studies, small chambers formed of stainless steel mesh are implanted subcutaneously. These chambers elicit a strong wound healing response and over the course of two weeks fibrous tissue fills the chamber. Addition of a collagenous matrix plus the platelet derived growth factor more than doubles the rate of cell infiltration into the chamber as well as doubling the deposition of collagen. These studies suggest that wound healing can be hastened or improved by the administration of matrix components, chemoattractants and mitogenic agents.

DEVELOPMENTAL BIOLOGY

Cartilage continues to be a major focus of our studies on developing tissues. Mutant strains of mice with altered cartilage have been studied. One of these genetically dwarfed animals has been shown to lack cartilage proteoglycan and another to lack cartilage collagen. DNA probes are being developed for the cartilage specific genes to allow detailed studies of chondrogenic expression in normal and diseased states.

Another mouse mutation we have worked on blocks biosynthesis of skin filaggrin. This prevents normal cornification of epidermal cells permitting adhesion of adjacent epithelia during development and causing multiple malformations.

A protein has been isolated from bovine testes that reversibly inhibits the differentiation of chondrocytes and other types of cells. A cross reacting protein is present in serum. It is possible that this factor maintains stem cells in an undifferentiated state.

Proteoglycans are ubiquitous constituents of all tissues. The cartilage proteoglycan is the best studied and seems to have a structural role. The heparan sulfate proteoglycan from basement membrane regulates permeability. We have now isolated other proteoglycans from bone and from other tissues. Immunological studies indicate that these are tissue specific. Their functions are under study.

LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00006-22 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on animal cell chemotaxis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Schiffmann, Elliott	Research Chemist	DB NIDR
Gleiber, Wayne	Postdoctoral Fellow	DB NIDR
Vasanthakumar, Geetha	Visiting Fellow	DB NIDR
Somerman, Martha	Staff Fellow	CIPC NIDR
Pencev, Dobimer	Guest Worker	DB NIDR
Mato, Jose	Guest Worker	DB NIDR
Garcia-Castro, I.	Guest Worker	DB NIDR
Hirata, F.	Res. Biochemist	LCS NIMH
Brownstein, M.	Res. Pharmacologist	LCS NIMH
Liotta, L.	Sr. Surgeon	LPP NCI
Huaso, R.	Visiting Scientist	LPP NCI
Manjunath, R.	Visiting Scientist	PR NICHHD
Mukherjee, A.	Res. Biochemist	PR NICHHD

COOPERATING UNITS (if any)
NCI, NIH; NIMH, NIH

LAB/BRANCH
Laboratory of Developmental Biology & Anomalies

SECTION
Connective Tissue Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

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SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the directed migration of cells (chemotaxis). We have defined peptides produced by bacteria that attract phagocytic cells. Now we have isolated and partially characterized materinls produced by tumors that are antichemotactic and possible involved in the evoidance of host rejection. These materials are peptides which may represent non toxic antiinflammatory substances.

A factor produced by encapsulated tumors is highly chemotactic for fibroblasts and may play a role in the formation of the tumor capsule. Chemoattractants may be involved in a variety of oral facial diseases including inflammatory diseases and oral cancer.

PHS-6040
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00009-21 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemistry and biosynthesis of connective tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Martin, George R.	Ch, Lab. Dev. Bio. & Anomalies	DB NIDR
Rohrbach, David	Postdoctoral Fellow	DB NIDR
Szarfman, Ana	Guest Worker	DB NIDR
Kleinman, Hynda K.	Research Chemist	DB NIDR
Crystal, Ronald	Ch. Pulmonary Branch	PB NHLBI

COOPERATING UNITS (if any)
Johns Hopkins University; NEI, NIH; NCI, NIH; University of Minnesota and Max-Planck-Institute for Biochemie

LAB/BRANCH
Laboratory of Developmental Biology & Anomalies

SECTION
Connective Tissue Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the formation, function and destruction of connective tissue components in normal and diseased states. Particular attention is directed toward collagen and proteoglycan. Current aspects of this project include (1) characterization of the matrix components in a tumor which produces basement membrane, (2) the role of collagen in diseases and (3) interaction of cells with collagen.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00024-16 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Developmental processes in genetically controlled malformations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Brown, Kenneth S.	Medical Director	DB NIDR
Harne, Leslie C.	Bio Lab Technician (Animal)	DB NIDR
Strong, David M.	Bio Lab Tech (Animal)	DB NIDR

COOPERATING UNITS (if any)
Howard University; University of Maryland; Univ. Washington, Seattle; NCI, NIH; NEI, NIH; NIAMDD, NIH; NIEHS, NIH; and USDA Poison Plant Laboratory

LAB/BRANCH
Laboratory of Developmental Biology & Anomalies

SECTION
Connective Tissue Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.05	.95	3.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Craniofacial defects due to specific genes in mice are the major subject of our investigations. Mutations that change the biochemistry of chondrocytes resulting in craniofacial skeletal disturbances and mutations that change the ability of epithelial cells to keratinize can result in malformations such as cleft lip and cleft palate, exancephaly and malocclusion. Other genes modify the ability of embryos to tolerate drug treatment, diets or environmental agents that cause similar malformations. These mouse genetic test systems are produced using uniform, highly inbred, strains and timed mating resulting in well defined stages of development with tissues at critical periods of susceptibility for in vivo or in vitro study. Cells from embryo tissues of appropriate genetic type and developmental stage are treated in culture using isotopically labeled precursors for specific structural molecules inorder to determine the mechanisms of gene action in developmeat and the mechanisms of teratogenesis. A high degree of genetic definition, available among mammals only in mice, is essential to assure molecular specificity of biochemical defects of development.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00025-16 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of connective tissue gene expression during development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Sobel, Mark	Research Associate	DB NIDR
Young, Marian	Postdoctoral Fellow	DB NIDR
Laurent, Maryvonne	Visiting Fellow	DB NIDR
Wuust, Jens	Visiting Fellow	DB NIDR
Kaul, Ravi	Visiting Fellow	DB NIDR
Oberbaumer, Ilse	Guest Worker	DB NIDR
Kuhn, Klaus	Fogarty Scholar	DB NIDR
Vogeli, Gabriel	Visiting Scientist	LVR NEI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Biology & Anomalies

SECTION
Connective Tissue Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.60	5.20	1.40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to understand the molecular mechanism by which genes for connective tissue proteins are differentially regulated and expressed during normal development and in disease states. We are using recombinant DNA techeology and conventional methods in nucleic acid biochemistry to study the structure and expression of genes coding for extracellular and cell associated structural proteins that are affected during development and in pathologic states. To study normal and abnormal cartilage development, we are examining the molecular basis for loss of phenotypic traits of chondrocytes after exposure to the thymidine analog 5-bromodeoxyuridine. Recombinant cDNA and genomic clones of collagen types I and II are being used to analyze the RNA and DNA of differentiated and dedifferentiated cartilage cells. We have also synthesized specific recombinant clones to study the coordinated regulation of the synthesis of the two component chains of type I collagen. In addition, a cDNA library is currently under conatruction to identify and study the genes for the basement membrane constituents, type IV collagen and its attachment protein, laminin.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00149-08 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Alterations in proteoglycans during abnormal development and disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Hassell, John	Research Biologist	DB NIDR
Kleinman, Hynda K.	Research Chemist	DB NIDR
Ledbetter, Steve	Guest Worker	DB NIDR
Tyree, Bernadette	Staff Fellow	DB NIDR
Termine, John	Research Chemist	LBS NIDR
Nilsson, Bo	Visiting Associate	LP NCI
Hascall, Vincent	Research Chemist	LB NIDR
Nakazawa, Kiyoshi	Visiting Scientist	CB NEI
Fisher, Larry	Guest Worker	LBS NIDR

COOPERATING UNITS (if any)

Emory University

LAB/BRANCH

Laboratory of Developmental Biology & Anomalies

SECTION

Craniofacial Development Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.93	3.00	4.93

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to understand the role that proteoglycans play in the growth and differentiation of craniofacial tissues and in other tissues undergoing similar developmental events. At present we are determining the mechanism of action of teratogens on chondrogenesis. We are also isolating and characterizing tissue specific proteoglycans and determining their function by studying abnormal development and disease.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00230-06 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of extracellular matrix proteins in tissue architecture and cell function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Kleinman, Hynda K.	Research Chemist	DE NIDR
Chandrasekhar, S	Visiting Fellow	DB NIDR
Woodley, David T.	Expert	DB NIDR
Martin, George R.	Ch, Lab. Dev. Biol. & Anomalies	DB NIDR
Drum, Ann M.	Clinical Associate	CB NIDR
Dubois-Dalcq, Monique	Research Biologist	LMG NINCDS
Liotta, Lance A.	Sr. Surgeon	LPP NCI
Robey, Pamela Gehron	Staff Fellow	RCD NEI
Rennard, Stephen I.	Research Associate	PB NHLBI

COOPERATING UNITS (if any) NEI, NIH; NCI, NIH; NHLBI, NIH; NINCDS, NIH; University of Minnesota; Veterans Administration Hospital, San Francisco

LAB/BRANCH

Laboratory of Developmental Biology & Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.01	3.00	3.01

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying a new class of matrix-derived glycoproteins which regulate embryogenesis and wound repair. In vitro, these glycoproteins control the assembly of the extracellular matrix and influence cell behavior by modulating cell adhesion, growth, migration, matrix production and differentiation. These proteins bind the cells to the matrix and specific attachment proteins exist for each cell type and matrix. It is also possible to control or suppress the growth and differention of certain cells by adding attachment glycoprotein not normally present in the tissue. For example, the addition of basement membrane-derived attachment glycoprotein inhibits the growth of fibroblasts and connective tissue cells and induces the outgrowth and differentiation of neurites from explants of human sensory spinal ganglia. Through these biological activities, these attachment glycoproteins can control which cells will populate a matrix during development and can effect repair reactions necessary to replace damaged tissue.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00253-05 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of cartilage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Varner, Hugh H.	Staff Fellow	DB NIDR
Hewitt, A. Tyl	Guest Worker	DB NIDR
DeLuca, Silvana	Senior Staff Fellow	LB NIDR
Hilsson, Bo B.	Visiting Scientist	LP NCI
Osborne, James C., Jr.	Senior Investigator	MD NHLBI

COOPERATING UNITS (if any)

University of Minnesota; Rutgers Medical School; Yale University

LAB/BRANCH

Laboratory of Developmental Biology & Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.43	3.00	2.43

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Factors that distinguish chondrocytes from other cells and factors active in regulating differentiating are under study. Chondronectin, the glycoprotein chondrocytes use to bind to matrix has been isolated from serum. Chondronectin is specific for chondrocytes and binds to type II collagen in the presence of proteoglycan. A new affinity purification using insolubilized glycosaminoglycan has been developed as a rapid purification for chondronectin.

A protein has been isolated from testes that causes chondrocytes to dedifferentiate and prevents certain other cell types from differentiating. A general role in maintaining stem cells in an undifferentiated state is suggested.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00275-04 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biological testing of fluoride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Martin, George R.	Ch. Lab. Dev. Bio. & Anomalies	DB NIDR
Brown, Kenneth S.	Medical Director	DB NIDR
White, Beverly	Cytogeneticist	LEP NIADDK
Kohn, Kurt	Ch., Lab. Mol. Pharmacology	LMPB NCI

COOPERATING UNITS (if any)

University of Minnesota; Litton Bionetics; and NCI, NIH

LAB/BRANCH

Laboratory of Developmental Biology & Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the action of fluoride in various systems used to detect clastogenic or mutagenic substances. To date fluoride has been examined in several systems used to detect mutagens and found to be non mutagenic. No effects on chromosome structure were noted in animals given widely different levels of fluoride. DNA repair after X-ray was unchanged by fluoride. No genetic effects of fluoride were noted in a recessive lethal test of fluoride on drosophila. The data indicate that fluoride has no mutagenic activity. Ongoing studies and reports of fluoride-effects on metabolism and growth in the literature are being monitored.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00330-01 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of attachment proteins in tumor cell metastasis and periodontal reattachment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Terranova, Victor	Research Associate	DB NIDR
Martin, George R.	Ch, Lab. Dev. Biol. & Anomalies	DB NIDR
Liotta, Lance	Ch, Lab. Pathology	LP NCI
Rao, C.N.	Visiting Fellow	LP NCI

COOPERATING UNITS (if any)

NCI, NIH; Georgetown University; Eastman Dental Clinic; University of Rochester; University of Michigan

LAB/BRANCH

Laboratory of Developmental Biology & Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.08	1.00	1.08

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is: (1) to study the interaction of human metastatic tumor cells with extracellular matrix components and (2) to determine whether laminin and fibronectin can promote the reattachment of periodontal connective tissue following chronic periodontitis. Particular emphasis is directed towards understanding the role of laminin in promoting the adhesion of metastatic tumor cells to the type IV collagen component of the subendothelial basement membrane.

The reattachment of soft tissue to teeth compromised by periodontal disease has long been an area of great interest. We have directed our efforts to studying the interaction of fibroblasts and epithelial cells with tooth structure utilizing laminin and fibronectin.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00331-01 DB

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Connective Tissue Cell Chemoattractants in Wound Healing and Fibrotic Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Grotendorst, Gary R.	Staff Fellow	DB NIDR
Pencev, Dobromir	Guest Worker	DB NIDR
Bleiberg, Ilan	Visiting Scientist	DB NIDR
Martin, George R.	Ch., Lab. Dev. Biol. & Anomal.	DB NIDR
Sodek, Jaro	Guest Worker	DB NIDR
McIver, Caroline	Guest Worker	LU NIADDK
Liotta, Lance	Act. Ch, Lab. Pathology	LP NCI
Barsky, Sanford	Expert	LP NCI

COOPERATING UNITS (if any)

NCI, NIH; NEI, NIH; NINCDS, NIH, University of Toronto, University of Minnesota; USUHS

LAB/BRANCH

Laboratory of Developmental Biology & Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.21	2.40	1.81

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have found that the platelet derived growth factor (PDGF) which is released from platelets in wounds is a potent chemoattractant for smooth muscle cells and for fibroblasts. These studies show that the chemotactic activity of PDGF is separate from its mitogenic activity. Addition of PDGF, collagen and fibronectin markedly stimulates connective tissue formation in models of wound healing. Various studies suggest that smooth muscle cells are brought to areas of injury by chemotaxis and the altered wound healing underlies fibrotic conditions. Transformed cells lose their ability to respond to normal wound hormones.

PHS-6040
(Rev. 2-81)

LABORATORY OF ORAL MEDICINE

The Laboratory of Oral Medicine studies the etiology and pathogenesis of both systemic diseases and diseases of the soft tissue of the oral cavity. Emphasis is on: (1) viral infections such as herpes simplex virus; (2) endocrine diseases, especially viral-induced diabetes mellitus; (d) autoimmune disorders; and (40 ulcerative and proliferative lesions of the oral cavity. The program is disease oriented and highly interdisciplinary. The Laboratory is made up of investigators who are trained in a variety of disciplines, including virology, immunology, pathology, cell biology, molecular biology, and clinical medicine and dentistry.

Over the last year, in-depth studies have continued on the projects discussed in previous annual report, with gratifying progress in the areas of herpes simplex virus, virus-induced diabetes and autoimmunity. Some of the contributions, especially in the latter area, have resulted in a major change in both the direction of our work, and the way we are thinking about autoimmunity.

This last year, the project on ulcerative lesions of the oral cavity was dropped because of the departure of Dr. David Wray, the principal investigator. We have now recruited Dr. Daniel Eskinazi, who holds both a D.D.S. and Ph.D. He will continue certain aspects of the project and start a new project on proliferative lesions of the oral cavity (especially papillomas and leukoplakia). To give strength to this project, it will be carried out in collaboration with the molecular biologists who will search for viral and host sequences and the immunologists who will look by monoclonal antibody techniques for "autoantibodies" that might be reacting with tumor antigens.

For the past two years, the pathology unit of the Laboratory has been without leadership. Dr. Floyd Taub has now been recruited to head up this unit. Dr. Taub is an M.D., trained in both pathology and molecular biology. He will first reorganize the pathology unit to make it more functional and to provide routine and special services for members of the Laboratory in histopathology, immunofluorescence and electron microscopy. A major problem in the past has been the difficulty in obtaining sufficient quantities of properly prepared human material for study by the various investigators in the Laboratory. With Dr. Taub's background and contacts in pathology, it should be possible to systematically organize the collection of material from various diseases on a more regular schedule. Once this is accomplished, the provisional plan is for Dr. Taub to spend the majority of his time studying autoimmune endocrine diseases using immunological and molecular approaches.

Three guest workers have spent or are spending a portion of their sabbaticals in our Laboratory. Dr. Mazagazu Horita, a physician from the University of Jikei in Tokyo, completed a study on immunoregulatory abnormalities in diabetes mellitus which will be published shortly in the Journal of Immunology. He has already returned to Japan. Dr. Soroku Yakihashi from the University of Hirosaki is an M.D. and is trained in pathology. He is spending part of a year with us working on antibodies to beta cells in human diabetes mellitus. Dr. Peter Wassmer just received his Ph.D. from the University of Basel. Dr. Wassmer will look by monoclonal antibodies for antigenic differences among insulin molecules in normal individuals and patients with diabetes.

The staff of the Laboratory continues to work well together on interdisciplinary projects, and we are fortunate to have a number of young, talented and aggressive investigators who are developing into independently recognized scientists. A number of the investigators are receiving invitations to deliver lectures at national and international meetings.

The service units of the Laboratory (i.e., tissue culture, histology, photography) and the Office of the Chief of the Laboratory continue to function very well. However, recently, Mrs. Edith Rian, who provided invaluable service in typing and editing manuscripts, became ill. Mrs. Jane Gascoyne was recruited to fill this void.

Over the last year, a number of new techniques have been introduced into the Laboratory and others have been extensively refined. The principal ones involve recombinant DNA and hybridoma technology. Specific methods include: (1) Northern blots for transferring RNA onto nitrocellulose paper for studies on transcription; (2) expression cloning with bacterial plasmids, especially engineered to carry promotors and protein initiation signals upstream from the cloning sites; (3) computer-aided mapping of DNA restriction fragments; (4) mRNA selection by preperative hybridization used to identify and clone genes expressing proteins of interest; (5) immunoprecipitation of *in vitro* translation products to assay for peptides synthesized *in vitro*, coded for by mRNAs selected on DNA restriction fragments; (6) direct recombinant DNA cloning using vector/host combinations that result in the elimination of all recombinant molecules that do not contain the DNA fragments of interest; this eliminates expensive and time-consuming screening of recombinants; (7) in situ hybridization for the screening of human tissue sections for the presence of viral DNA and/or transcripts (mRNA); (8) affinity chromatography for the purification of antigens recognized by monoclonal autoantibodies; (9) Western blots to identify proteins recognized by autoantibodies; crude or purified

antigen preparations ar electrophoresed on polyacrylamide gels, transferred onto nitrocellulose, and stained with antibody and peroxidase-conjugated anti-immunoglobulins; (10) isoelectric focusing - used both analytically and preparatively to separate proteins (antigens, monoclonal antibody) according to their isoelectric points; (11) new methods for the preparation of human beta cell cultures; (12) introduction of BuDR or 8-azoguanine drug markers into continuous cell lines to be used in cell to cell fusion experiments; (13) a variety of ELISA techniques for measuring antibodies and hormones; (14) development of immunoperoxidase assays to measure various types of autoantibodies; and (15) development of human hybridoma methods for studying monoclonal antibody production.

Collaboration continues or has been initiated with investigators from other Laboratories within NIDR, with investigators from other Institutes at NIH, and with colleagues at various universities. Active collaboration projects include: (1) long-term complications of diabetes (NEI); (2) alteraions in the synthesis of basement membrane in diabetic mice (Laboratory of Developmental Biology and Anomalies, NIDR); (3) cultivation of human insulinoma and gastrinoma cells (Diabetes Branch, NIADDK); (4) virus-induced diabetes in autoimmune New Zealand mice Arthritis and Rheumatism Branch, NIADDK); (5) decresed bone formation and mineralizaiton in virus-induced diabetes (Laboratory of Biological Structure, NIDR); (6) ribonuclease T_1 mapping of viral RNA by two-dimensional electrophoresis (NINCDS;) (7) sequencing of viral proteins (City of Hope Research Center, California); (8) studies to identify the polypeptides of Coxsackievirus that are involved in neutralization using monoclonal antibodies to Coxsackie B4 (Department of Microbiology, Hahnemann Medical College); (9) the effect of virus-induced changes on the luxury function (but not the Jolla); (10) studies on the epidemiology of Coxsackie B4 variants in different geographical locations and at different times with monoclonal antibodies (Department of Microbiology, University of Rochester); (12) studies on HSV sequences in ganglia and human brain (Department of Neuropathology, University of Southern California); (13) cloning and expression of HSV sequences (City of Hope Research Center, California, and the University of Tennessee); (14) a variety of studies on interferon with investigators from Walter Reed, Johns Hopkins, University of Pennsylvania, and several of the Institutes at NIH.

IMPORTANT FINDINGS

Some of our more important finding since last year's annual report are summarized below.

1. Herpes simplex virus (HSV) causes lifelong infections of the central and peripheral nervous systems in mice and humans. Our efforts to obtain an understanding of the biochemical basis of latency have continued this year with several major advances. We have now demonstrated by three independent approaches that the viral genome is integrated into the DNA of latently infected trigeminal ganglion cells of mice. Definitive confirmation awaits the isolation and purification of covalently joined virus-cell DNA molecules. Since one would expect to find only low concentrations of joint molecules in ganglia, recombinant DNA techniques are being used to clone and amplify the joint molecules. Several phage recombinant clones containing viral-like DNA were obtained in our initial experiments. Detailed studies showed that the DNA in these clones was not viral in origin, but exclusively derived from a region of the mouse genome that has a high degree of sequence homology with a short stretch of the viral genome. We have mapped both mouse and viral DNA regions and have determined that the homologous sequences are localized within a 1000 base pair fragment very near the right terminus of the HSV genome. Furthermore, we have found the region of homology to be present in uninfected human DNA. The possibility that these sequences represent pre-integraiton sites is being explores, as well as their possible involvement in HSV-induced transformation of tissue culture cells.

2. Progress has been made in our long-range goal of developing subunit vaccine against HSV. Recombinant DNA clones contining the region of the viral genome coding for the viral B2 glycoprotein have been obtaind. Further restriction enzyme mapping, subcloning and *in vitro* protein synthesis experiments have located the gene coding for this major viral antigenic determinant.

Recently, the second phase of the project, the cloning of the B2 gene in an expression plasmid in order to produce large amounts of the polypeptide, was initiated. Unfortunately, the complete B2 protein seems to be highly toxic for bacterial cells, since all the clones proved to be unstable and died when their propagation was attempted. It is very likely that instability results from the presence of "killer" hydrophobic signal sequences in the polypeptide, as it seems to be in at least two other cases, (i.e., the expression cloning of vesicular stomatitis virus G antigen and hepatitis B antigen and hepatitis B antigen). We will now start a series of experiments geared to the expression cloning of short pieces of the B2 gene lacking regions coding for "killer" sequences. We will screen the clones radioimmunologically for the presence of regions of the B2 polypeptide by anti-B2 antibodies.

3. Two new projects were initiated this year, and both have been moving at a good pace. First, a search for viral mRNA in latently infected cells by in situ hybridization has resulted in the demonstration of viral

transcripts in cytologic sections of latently infected mice ganglia. The areas where active mRNA synthesis occurs are clustered and few in number. We expect in the near future to extend these studies to brain stems and hemispheres of mice and to nervous system tissue of humans. Second, we have started a long postponed search for viral DNA in human neural tissues by Southern blotting analyses. We have been unable to detect viral-like DNA fragments at an extremely low frequency in several ganglionic DNA samples, but cannot at present unambiguously prove that they are of viral origin, since they may represent regions of virus-cell homology (see # 1, above). Further steps in their analysis and purification, and eventually cloning by recombinant DNA techniques, will be undertaken during the next year.

4. Interferons (IFNs) have antiviral and immunoregulatory activities. Previously, we showed that IFN-alpha was present in the circulation of patients with certain autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and Sjogren's Syndrome (AIDS). In other experiments, antibody to interferon was found in two patients with lupus erythematosus. These interesting observations are now being pursued.

The role of viruses in human autoimune disorders is poorly understood. Alterations of the host's immune response during viral infections have been observed with a variety of viruses. During the past year, we initiated studies on the relationship between cytomegalovirus (CMV) and immunologic disorders. We did a sero-epidemiological survey on the prevalence of anti-viral antibody in patients with autoimmune and lymphoproliferative diseases. The data, although still very preliminary, suggest that recent CMV infections are frequent in patients with systemic lupus erythematosus and Sjogren's syndrome. We are now trying to isolate viruses from patients with immunoregulatory disorders such as lupus erythematosus, Sjogren's syndrome and AIDS.

5. Advances also have been made in our studies on virus-induced diabetes. The M variant of encephalomyocarditis (EMC) virus infects and destroys insulin-producing beta cells in the pancreas of mice. About two years ago, we found that our EMC virus pool contained not one virus, but at least two variants: one highly diabetogenic (designated D) and the other nondiabetogenic (designated B). The separation of our virus pool into the D and B variants has made several new types of experiments possible.

A. First, molecular biological approaches were used to distinguish the B and D variants. The genomes of the diabetogenic and nondiabetogenic variants were analyzed by nucleic acid hybridization and RNA fingerprinting. cDNAs of EMC-D and EMC-B were prepared and hybridized to the RNAs of the D and variants. Hybridization and thermal elusion profiles failed to show any differences between the RNAs of these two variants. However, fingerprints of the T_1-digested RNAs revealed at least one oligonucleotide, 20-25 bases long, that was present in the diabetogenic D variant, but was missing in the nondiabetogenic B variant. Although this oligonucleotide has not yet been mapped to a specific region of the genome, it is possible that it is the part of the genome that is responsible for the differences in the biological activities of the two variants.

B. Second, the separation of our virus pool into the D and B variants has made it possible to study some of the long-term complications of diabetes. The D variant, in the absence of the B variant, produces far more severe and prolonged diabetes than the original (mixed) virus pool. In humans with diabetes, the risk of developing blindness is increased 25-fold, kidney disease 17-fold, heart disease 2-fold, and life expectancy decreased by one-third. Over the last year, we studied mice that had been infected with the D variant and that were diabetic for six months. The kidneys of the diabetic animals showed both diffuse and nodular type of glomerulosclerosis. Electron microscopy revealed a two- to four-fold increase in the thickness of the glomular basement membrane. These findings are typical of those seen in humans with the Kimmelstiel-Wilson type of diabetic kidney disease. In addition to the glomerular changes, the diabetic animals showed some of the same type of early ocular changes found in retinal vessels (e.g., a decrease in periocytes) of patients with diabetes mellitus. In addition, there was a four- to six-fold increase in mortality of the highly diabetic animals as compared to control animals. Thus, the animal model is complete in the sense that the virus can produce both the early metabolic changes and at least some of the long-term complications of insulin-dependent diabetes.

C. Third, it was found that the nondiabetogenic B variant was antigenically similar to the diabetogenic D variant. Over the last year, we demonstrated that immunization of mice with the nondiabetogenic B variant completely prevented the development of diabetes in mice subsequently challenged with the diabetogenic D variant. Thus, at least in mice, virus-induced diabetes can be completely prevented by a live attenuated vaccine.

6. Diabetes mellitus is a heterogeneous group of diseases, and even the insulin-dependent form of diabetes appears to have more than one cause. Both environmental insults (e.g., viruses) and/or the host's

immunological response to foreign or self antigens have been implicated. In fact, immunological abnormalities have been found in some patients with insulin-dependent diabetes mellitus (IDDM). Autoantibodies reacting with cytoplasmic antigens in pancreatic islet cells have been detected in up to 85% of newly diagnosed IDDM patients, tapering off to about 20% at the end of two years. Over the last couple of years, we also found antibodies that reacted with antigens on the surface of islet cells. These antibodies, at least under *in vitro* conditions, can lyse cultured rat beta cells in the presence of complement. During the past 12 months, we looked for additional evidence of immunological abnormalities. We found an alteration in the ratio of phenotypic helper to suppressor cells as evaluated by specific monoclonal antibodies. We showed that the helper/suppressor cell ratio was significantly increased in patients with IDDM of less than two months duration and then gradually returned to normal. Despite the alteration in the helper/ suppressor cell ratio, there was no evidence for polyclonal activation as measured by the number of immunoglobulin-secreting, plaque-forming cells in the peripheral blood. However, there was a significant increase in the number of immunoglobulin-secreting, plaque-forming cells in the patients suffering from both IDDM and Hashimot's thyroiditis (i.e., polyendocrine disease). These and other findings suggest that subtle changes in the immunoregulatory system occur during the early stages of IDDM.

7. Good progress has been made in the area of viral receptors. Certain viruses, such as the cardioviruses and the group B Coxcsackieviruses, induce a broad spectrum of clinical syndromes. The presence or absence of receptors on the surface of cells is known to determine the host-range. This year, we have obtained evidence that for at least two strains of cardiovirus, the D variant of EMC virus and mengovirus, receptor specificity determines which tissues will be susceptible to infection. Although EMC virus and mengovirus cannot be distinguished antigenically by hyperimmune sera, EMC virus induces diabetes, whereas mengovirus induces a rapidly fatal encephalitis. Receptor blocking experiments were done using a neuroblastoma cell line and purified labeled and unlabeled viruses. Unlabeled mengovirus blocked the binding of radiolabeled mengovirus, but not the binding of radiolabeled EMC virus. Conversely, unlabeled EMC virus blocked the binding of radiolabeled EMC virus, but not the binding of radiolabeled mengovirus. Other experiments showed that the attachment of mengovirus to neuroblastoma cells was 5 to 10 times faster than the attachment of EMC virus, whereas both viruses bound equally well to non-neuronal cell lines. *In vitro* experiments showed that neuroblastoma cells were about 10 times more susceptible to mengovirus infection than EMC virus infection. It is concluded from these and other studies that EMC and mengo are related, but distinct viruses that bind to different receptors on the cell surface. These findings suggest that the existence of variants in a virus pool, each with its own receptor specificity, may determine the type of clinical disease produced in exposed individuals.

8. We have also completed our study of the induction and modulation of virus receptors. Using murine lymphoid and myeloid cells, we found that receptors for EMC Virus can be induced by culturing receptor-negative lymphocytes in medium containing antigens. The induction occurred with both T and B lymphocytes, and a requirement for prior DNA synthesis was shown. Moreover, following the induction of receptors, the stimulated lymphocytes became susceptible to EMC virus infection. Other experiments showed that only a small subpopulation of lymphocytes were inducible for virus receptors. We have also shown that during different stages of cell growth and differentiation, virus receptors can be modulated. Changes in receptor expression of up to 10-fold were found when cells were tested at different phases of growth or after treatment with agents that induce differentiation *in vitro.* Thus, the changes in receptor expression, especially on cells involved in the immune response, may contribute to the susceptibility of the host to certain viral infections.

9. A complex antigen such as a virus, a hormone or a cell component, is made up of numerous antigenic sites; a change in a single site may affect the cell tropism of a virus or the biological effectiveness of a hormone. Monoclonal antibodies, produced by a hybrid between a nonreplicating specific antibody-producing lymphocyte and a mutant myeloma cell, can discern changes in a single determinant. We have, therefore, isolated hybridomas that produce specific antibodies as probes of viral and autoimmune diseases.

The Coxsackie B virus group consists of six serotypes (B1-6) that are antigenically distinct. In patients, the Coxsackie B4 serotype can produce a variety of clinical diseases (e.g., pleurodynia, respiratory illness, meningitis, myocarditis, orchitis and diabetes). It is not known whether these diseases are due to a chance infection of a particular organ or due to variants of Coxsackie B4 that have a different tissue tropism. The reference hyperimmune sera used throughout the world for over 15 years to type clinical isolates do not identify variants that may exist within each of the serotypes. Therefore, in an attempt to identify variants of Coxsackie B4 serotype, we have prepared a panel of 70 monoclonal antibodies arising from 22 different hybridomas. Initially, 18 monoclonal antibodies were characterized as to their subclass, neutralization titer, and reactivity pattern with recent clinical isolates of

Coxsackie B4 virus. Using these monoclonal antibodies, we have identified a large number of antigenic variants of Coxsackie B4 and have shown that there are major antigenic differences among naturally-occurring isolates. Furthermore, using these antibodies as selecting agents, we find that the frequency of antigenic mutants may be as high as 10^{-4}. Identification of a large number of variants with major antigenic differences points to the possibility that the different clinical pictures seen in this viral infection might be due to variants that have different tissue tropism. Our future studies involve characterizations of a large number of clinical isolates obtained from patients with different diseases. If a correlation between antigenic variation and disease pattern could be established, then monoclonal antibodies might be used to classify subtypes of Coxsackie B4 virus and analyze variants from different year and from different geographic locations.

10. There are a number of human diseases for which the etiology is unknown, but which have an autoimmune component. In some of these diseases such as myasthenia gravis, the autoimmunity is restricted, for example, to specific receptors. But in other diseases such as systemic lupus erythematosus, the autoimmune response is broad, involving many different organs. Similarly, in patients with diabetes mellitus, autoantibodies have been found that react with pancreatic cell surface and cytoplasmic antigens, anterior pituitary, thyroid, and gastric mucosa. Autoantibodies directed against DNA, RNA and lymphocytes also have been reported.

The nature of autoantigens and the events that trigger autoimmune response are unknown, but viruses have been suggested as one of the possible causes. Recently, we showed that reovirus type 1 infection of SJL/J mice results in polyendocrine disease characterized by mild diabetes mellitus and growth retardation. Autoantibodies directed against normal pancreas, pituitary and gastric mucosa were found in the sera of these animals. However, the specific role of these antibodies in the disease process has been hard to establish because these antibodies are difficult to obtain in large quantities and in a relatively pure form. To accomplish this end, we used spleen cells from reovirus-infected mice to develop hybridomas that produce autoantibodies. With this technique, we now have obtained a panel of monoclonal antibodies that react with a variety of normal tissues. We have obtained 23 monoclonal autoantibodies that react with different subpopulations of cells in the anterior pituitary. Some of these antibodies are directed against growth hormone. The second most frequently isolated autoantibodies (a total of 13) are directed against the cells in the periphery, but not in the central area of the islets of Langerhans. Some of these autoantibodies are reactive with glucagon. In contrast, a single autoantibody has been obtained that reacts with the central portion of the islets and also with rat insulin. Also, we have obtained 5 hybrodomas that react with nuclear antigens. Nine monoclonal autoantibodies have been obtained that react with cells in gastric mucosa, but their specificities still are not known. Further studies will be aimed at answering some fundamental questions. For example, it should now be possible to determine whether different individuals with a specific disease develop autoantibodies against the same molecules, and if so, whether they are directed against the same antigenic determinant(s). Studies are underway to isolate and identify some of the autoimmunogens, the nature of which are unknown. The use of hybridomas to isolate and study autoantibodies is now being extended in our Laboratory to human diseases.

LABORATORY OF ORAL MEDICINE

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00080-09

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Diseases of the Pancreas and Salivary Glands: Virus-Induced Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Yoon, Ji-Won	Research Microbiologist	LOM, NIDR
Onodera, Takashi	Visiting Associate	LOM, NIDR
Suzuki, Hoshibumi	Visiting Fellow	LOM, NIDR
Doberson, Michael J.	Staff Fellow	LOM, NIDR
Ray, Usharanjan	Visiting Associate	LOM, NIDR
Aulakh, Gurmit S.	Expert	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)
Dr. Fredda Ginsberg-Felner, Mt. Sinai School of Medicine

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.60	4.60	5.00

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mice infected with the diabetogenic D variant of EMC virus developed some of the long-term complications of diabetes, including diffuse and nodular glomerulsclerosis, an increase in the thickness of the glomerular basement membrane, mild retinal changes, and a four- to six-fold increase in mortality. Thus, animals infected with the diabetogenic variant of EMC virus develop both the early metabolic changes and at least some of the long-term complications of insulin-dependent diabetes.

The nondiabetogenic B variant of EMC virus is antigenically similar to the diabetogenic D variant of this virus. Immunization of mice with the nondiabetogenic B variant completely prevented the development of diabetes in mice subsequently challenged with the diabetogenic D variant. Thus, at least in mice, virus-induced diabetes can be completely prevented by a live, attentuated vaccine.

Reovirus type 1 triggers an autoimmune polyendocrine disease characterized by transient diabetes and growth retardation. This syndrome can be prevented by immunosuppression.

PHS-6040 (Rev. 2-81)

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00123-09

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Herpes Simplex Virus: Latency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Puga, Alvaro	Expert	LOM, NIDR
Cantin, Edouard M.	Expert	LOM, NIDR
Aulakh, Gurmit S.	Expert	LOM, NIDR
Cremer, Kenneth J.	Sr. Staff Fellow	LOM, NIDR
Gomez-Marquez, Jaime	Visiting Fellow	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to study the molecular events leading to the establishment of herpes simplex virus latent infections in the nervous systems of experimentally infected mice and of humans. The viral genome has been found in an integrated state in the trigeminal ganglion DNA of latently infected mice and various regions of DNA homology between viral and host (mouse and human) have been cloned from the mouse genome. Their possible involvement as preintegration sites is being studied. Viral DNA sequences have been found in ganglia of normal humans and in situ hybridization techniques have been used to detect the presence of viral transcripts.

The gene coding for one of the major antigenic determinants in the virus surface has been precisely mapped and its cloning in an expression plasmid is being attempted.

PHS-6040 (Rev. 2-81)

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00219-06

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Interferon, Autoimmunity and Viral Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Hooks, John J.	Microbiologist	LOM, NIDR
Shimizu, Fumio	Visiting Associate	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)
Dr. B. Detrick-Hooks Walter Reed Army Hospital
Dr. A. Levinson Univ. of PA, Phila., PA
Dr. H.M. Moutsopoulos Univ. of Ioannina, Greece Dr. J.L. Decker ARB, NIAMDD

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of viruses and interferon (IFN) in human immunoregulatory disorders and in normal immune reactivity is presently under investigation. These studies demonstrate that IFN is frequently present in patients with autoimmune disorders and in selected instances, antibodies to IFN can also be demonstrated in these patients with active disease. Moreover, these studies show that defective IFN gamma production in vitro is frequently associated with certain lymphoid malignancies and autoimmune disorders. Mechanisms of regulation of immune reactivity by human IFN have also been delineated. Studies have been initiated to investigate the role of viruses, especially cytomegalovirus, in human immunoregulatory disorders.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00255-04

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Receptors, Membranes and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

McClintock, Patrick R.	Staff Fellow	LOM, NIDR
Shimizu, Fumio	Visiting Fellow	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)
Kahn, C. Ronald D8, NIAMDD

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The expression of cell surface receptors for viruses and hormones is being studied using animal models of human diseases. The induction and modulation of receptors for encephalomyocarditis virus and insulin have been studied, and the results have shown that virus and hormone receptors can be regulated in response to a variety of in vivo and in vitro stimuli. Evidence that receptors can determine tissue tropisms in vivo has been found using variants of encephalomyocarditis and mengovirus. Results of insulin binding studies have shown that under certain conditions, insulin receptor activity of leukocytes may be altered while receptor activities of other tissues remain normal. Thus, for some patients, especially those suffering from immunologically-mediated diseases, results of insulin binding assays using leukocytes may not truly reflect insulin receptor activity on other cell types.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00309-02

PERIOD COVERED
October 1, 1981 - September 20, 1982

TITLE OF PROJECT (80 characters or less)
Hybridomas: Probes to study viral and other diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Haspel, Martin V.	Sr. Staff Fellow	LOM, NIDR
Prabhakar, Bellur S.	Staff Fellow	LOM, NIDR
Onodera, Takashi	Visiting Associate	LOM, NIDR
Satoh, Jo	Visiting Fellow	LOM, NIDR
Shimizu, Fumio	Visiting Fellow	LOM, NIDR
Kende, Meir	Expert	LOM, NIDR
Yoon, Ji-Won	Research Microbiologist	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.80	5.30	3.50

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoclonal antibodies have been developed against Coxsackie B4 and Encephalomyocarditis (EMC) viruses. These antibodies have been used to identify antigenic variations among these viruses. Attempts are underway to see whether there is a correlation between tissue tropism of these viruses and expression of certain antigenic determinants.

Monoclonal autoantibodies against pituitary, stomach, intestine, pancreas, etc. have been obtained from splenic lymphocytes of reovirus type 1 infected mice. These autoantibodies will be used to examine the spectrum of autoimmune responses in polyendocrine disease. Hybridoma technology is also being used in an attempt to obtain stable cell lines capable of producing monoclonal hormones.

PHS-6040
(Rev. 2-81)

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

The Clinical Investigations and Patient Care Branch functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (1) to conduct high quality, clinically oriented dental research programs; (2) to encourage and to provide support, consultation and facilities for clinical research activities of other Branches and Laboratories within the Institute; (3) to offer consultation on oral and dental problems to other Institutes; (4) to render clinical care to specified patients of the Clinical Center; and (5) to sponsor a training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research oriented dental clinicians.

The past year has been one of considerable transition for this Branch. Organizationally the structure of the Branch now includes two active, viable sections, the Clinical Investigations Section and the Patient Care Section. On January 1, 1982 Bruce Baum assumed the position of Clinical Director, Chief of the Branch and of the Clinical Investigations Section. Michael Roberts, Chief of the Patient Care Section, assumed his position in August, 1981 and has thus completed his first year as the individual responsible for day to day operation of the dental clinic. Substantial efforts have been made so that the Branch can attain its stated goals and provide the Institute with excellence in its intramural clinical programs. All of our personnel recognize that we are involved in a particularly challenging endeavor. Cooperation, assistance and understanding have been practiced to a high degree and we are enthusiastic about our future activities.

PATIENT CARE SECTION

The Patient Care Section conducts the daily operation of the NIDR clinic and as such is the focus of clinical oral and dental health concerns at NIH. In general, the staff of the Section has remained stable during the past year. The section provides a wide range of diagnostic consultative services to NIH clinical care programs. The section has continued to become more involved with the medical staff of the various Institutes and of the Clinical Center. Staff dentists and dental hygienists routinely participate in medical rounds and patient discussions, thus integrating oral health care concerns to total patient management. This section also is responsible for providing clinical training and development for Dental Staff Fellows.

This year a Dental Clinic Operations Manual was written and is being utilized to improve and standardize clinical operations and procedures. The manual decribes policies, forms and standard operating procedures within the Dental Clinic. A peer review system of oral health care has also been established to monitor patient services and to insure a high quality of dentistry.

In the past the compiling of dental epidemiology and patient care data has been a laborious manual task. This year a computer terminal was purchased for use in the Dental Clinic and a program written by the Scientific Systems Section of the Intramural Research Program, NIDR, to compile epidemiology and patient services data. Data are stored and identified by individual health care provider, Institute and medical diagnosis. This data collecting system was implemented in May, 1982, and is proving to be a valuable management tool with outstanding research potential.

The Patient Care Section has established an affiliation with the Baltimore College of Dental Surgery, University of Maryland. This collaboration will provide senior dental hygiene and dental students an opportunity to participate in alternative practice settings beyond those offered in the dental school core curriculum. The arrangement provides our staff with certain academic clinical dental teaching responsibilities and should provide a professional development opportunity. Section staff members will serve as preceptors in the program and have received faculty appointments as Clinical Field Instructors with the University.

In addition many of the staff members have been actively involved in both clinical and laboratory "related" research projects. These include the following studies: (1) epidemiological and clinical parameters of jaw lesions in Burkitt's Lymphoma in the American population; (2) the mechanism of adherence to tooth root surfaces of the gram negative, filamentous gliding bacteria, cytophaga; (3) qualitative differences in microbial populations present in sub-gingival plaque; (4) non-surgical treatment modalities for periodontal disease; (5) herpes zoster and the development of extensive facial and alveolar bone pathology;(6) oral developmental defects in Albright's Hereditary Osteodystrophy; (7) biochemical characteristics of specific bone proteins; (8) oral anomalies associated with Reiger's Syndrome; (9) the effects of steroids on post-operative inflammatory response following extraction of third molars; and (10) developing methodology for using cell surface markers as an adjunct to classical morphological criteria for cytological diagnosis.

CLINICAL INVESTIGATIONS SECTION

The Clinical Investigations Section was established as a major step in bringing to NIDR an active, high quality clinical-problem oriented, dental research program. The staff of this Section has been assembled both from persons new to the Institute as well as individuals who have been transferred to the Section from existing Institute Staff. Formally the Section was activated on January 1, 1982 but only came together as a coherent unit about July 1, 1982. During this period the Section put considerable effort into developing its space allotment in the Clinical Center into modern, functioning biological laboratories. From laboratory design to overseeing construction efforts, to equipping empty space, the Section has created research facilities appropriate for this important investigative endeavor.

While the new laboratories were being constructed, the Section spent much of the year apart, under less than ideal laboratory, and intellectually interactive conditions. Despite these handicaps, the Section has succeeded in continuing established research activities as well as beginning several new projects. As initially established, the Section has two major general subject areas of interest: (1) understanding the regulation of salivary gland function, and salivary secretion; (2) studying specific factors influencing the development of periodontal diseases, as well as understanding endogenous protective defense mechanisms versus these conditions.

Salivary studies focus on understanding biochemical mechanisms of saliva formation and alterations in these processes occurring during normal aging and certain disease states. Saliva is of critical importance to the maintenance of normal oral and dental health. Many of the oral health problems of older adults likely are the result of either specific age-related alterations in salivary gland function or alterations in function secondary to diseases and therapeutic treatments common to the elderly situation. Using *in vitro* cell models, considerable progress has been made in understanding neurotransmitter regulation of saliva formation. The aged rat parotid gland was shown to display an altered α-adrenergic physiologic response (K^+ efflux) in the presence of normally functioning α-adrenergic receptors. This paradigm is now being used as a probe of α-adrenergic receptor signal transduction mechanisms. Thus far it has been demonstrated the functional deficit is likely located just distal to the α-adrenergic receptor, but prior to associated phospholipid turnover and Ca^{++} mobilization steps. Further, it was shown that this defect could be corrected if the receptor was bypassed and K^+ efflux induced by the Ca^{++} ionophore A23187.

Other *in vitro* studies have examined intermediary metabolic consequences of adrenergic stimulation of salivary glands. Secretion is an energy dependent process and we have examined requirements and characteristics of α-adrenergic stimulation of glucose oxidation in the rat parotid gland. Also adrenergic agonists have been shown to have profound effects on protein production and processing in rat submandibular glands *in vitro* in addition to their well known induction of secretion.

An *in vivo* model of studying rat parotid and submandibular gland saliva secretion has been utilized. Basic studies performed have demonstrated the similarity of contralateral submandibular gland secretions and the stability of these secretions over a longitudinal experimental period. Several criteria have been followed including flow rate, $[K^+]$, $[Na^+]$, and the contents of total protein, lactoperoxidase and alkaline protease.

The other general group of laboratory studies by this Section have focused on phenomena and events related to periodontal diseases. One series of investigations have examined the role of free radicals (superoxide, hydroxyl radicals) in immune response cells. Particular attention has been paid to defining endogenous antioxidant mechanisms. New methods have been developed to measure extracellular free thiol concentrations in cell cultures to study the role of thiols as protective antioxidants. It was obseved that cells exposed to various concentrations of mixed disulfides divide and function in proportion to their capacity to cleave these disulfides to free extracellular thiols. This activity requires either a mitogenic or antigenic stimulus. T and B lymphycytes and macrophages were capable of this activity. High oxygen tensions, or ixidizing reagents, decreased cell proliferation only after and in proportion to decreasing thiol content. Additional studies have implicated that γ-glutamyl transpeptidase is the membrand-bound enzyme responsible for this antioxidative role of immune cells, in inflammation.

Another area of investigation has been the study of mechanisms regulating osteogenic processes. A bone specific protein, which displays chemotactic activity for osteoblasts, has been partially purified from extracts of human and rat bone. The protein is heat labile, trypsin sensitive, displays a Mτ between 40,000-70,000 daltons and was not observed in extracts of enamel or dentin. Other non-collagenous proteins found in bone, (osteocalcin, osteonectin, α-^{2}S glycoprotein) were not chemoattractants. The chemotactic protein had no effect on osteoblast attachment to Type I collagen. Since the initial events in fracture healing and repair of alveolar bone destruction include the migration of cells to the healing site, chemotactic factors, such as that

demonstrated in these studies, likely are of considerable import in stimulating new bone formation.

Studies have also been initiated to evaluate the role of the gram negative oral microorganism *Cytophaga* in the initiation and progression of periodontal diseases. One approach taken has been to develop a series of monoclonal antibodies with varying specificity toward human and isolates of *Cytophaga* and the related species *Capnocytophaga*. Thus far these studies have been used to demonstrate the extensive heterogeneity of these species in the oral cavity. In addition an *in vitro* model has been developed to study the mechanism(s) of attachment of *Cytophaga* to collagen, a major structural component of teeth and periodontal tissues. Attachment of *Cytophaga* to Type I collagen was enhanced by fibronectin at low concentrations ($<$ 10 μg/ml), while another attachment factor (laminin) and bovine serium albumin were without effect. Whole serum and fibronectin-depleted serum significantly inhibited *Cytophaga*-attachment to type I collagen, suggesting the presence of factors capable of modulating the pathologic potential of these bacteria.

Human research studies have thus far focused on problems related to oral health in the aged. Salivary gland functional studies have evaluated electrolyte secretion from stimulated parotid glands. The release of Ca^{++} and K^{+} was similar in different aged persons, independent of flow rate adjustments. The secretion of Na^{+} however was significantly diminished in older persons, reflecting increased Na^{+} reabsorption by ductal cells. Two separate statistical approaches were utilized to study this question, since Na^{+} secretion is significantly influenced by flow rate; analysis of covariance (flow rate as a covariate) and regression analysis. Both methods of analysis indicated a highly significant reduction in Na^{+} output with increased age among older males, while more modest reductions were seen with females.

We have also evaluated certain oral motor functions in different aged persons. These studies have focused on masticatory muscle performance, postural functions (lips, tongue) and muscles involved in swallowing. Several specific alterations in the oral motor apparatus were observed which appeared independent of health status. Such changes would affect daily-life oral motor performance and thus could influence the quality of life experienced by older persons.

Studies on gustatory function across the human life span have continued. Efforts have focused on the refinement of psychophysical methods for evaluating suprathreshold measures of taste intensity. Persons from grade-school through old age have been examined. Alterations occurring in the ability to taste have been quality specific and generally modest in extent.

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00096-08 CI

PERIOD COVERED: October 1, 1981- September 30, 1982

TITLE OF PROJECT (80 characters or less): Studies on Microbiologically Monitored and Modulated Periodontal Therapy in Humans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Rams, Thomas	Staff Fellow	CIPCB	NIDR
Sarfatti, David	Staff Fellow	LMI	NIDR
Krichevsky, Micah	Chief,MSS	MSS	NIDR
Rogosa, Morrison	Scientist Emeritus	MSS	NIDR

COOPERATING UNITS (if any): Paul H. Keyes, International Dental Health Foundation, Reston, Virginia

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Patient Care Section

INSTITUTE AND LOCATION: National Institute of Dental Research

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies demonstrate the value of developing microbiological criteria for use in periodontal diagnosis and for evaluating the efficacy of therapeutic measures. Phase-contrast microscopy was employed to evaluate the subgingival microflora at chairside in persons with adult periodontitis, juvenile periodontitis, marginal gingivitis and excellent periodontal health. Microbial risk factors for periodontal disease were recognized and a Microbial Index for scoring phase-contrast microscopic observations was formulated. Antimicrobial therapeutic methods were evaluated ($NaHCO_3$, NaCl, $MgSO_4$ and tetracycline) in a blind fashion for use in augmenting the antimicrobial effects of scaling and root planing in periodontal treatment. Various patient groups were treated with antimicrobial therapy of scaling, root planing, subgingival application of antimicrobial agents, and systemic administration of antibiotics in the presence of advanced lesions. All groups had dramatic reductions in pocket depth and increases in attachment levels following the elimination of microbial risk factors. Teeth with hopeless prognoses under conventional standards stabilized in most cases with clinical improvements.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DE 00212-06 CI

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less): Taste and Its Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Weiffenbach, J.M.	Psychologist	NIDR
Cowart, B.J.	Psychologist	NIDR

COOPERATING UNITS (if any): Laboratory of Behavioral Science, NIA

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The selection and refinement of psychophysical methods appropriate to the seperate measurement of various aspects of taste perception is a primary and continuing concern of this project. Normal variation in taste perception with chronological age is investigated with procedures which quantify not only the taste detection threshold but also the intensity and pleasantness of the taste experience elicited by stimuli at more commonly encountered intensity levels. Naturally occurring anomalies and therapeutically induced changes in taste are similarly investigated.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DE 00320-02 CI

PERIOD COVERED: October 1, 1981-September 30, 1982

TITLE OF PROJECT (80 characters or less): Survey of Jaw Lesions in Burkitt's Lymphoma in the NIH Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Donahue, Agnes H.	Staff Fellow	CIPCB	NIDR
Sariben, Eric	Clinical Associate	POB	NCI
McGrath, Ian T.	Senior Medical Staff	POB	NCI

COOPERATING UNITS (if any): Pediatric Oncology Branch, National Cancer Institute

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Patient Care Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS Chart review

SUMMARY OF WORK (200 words or less - underline keywords)

The epidemiological and clinical parameters of jaw lesions in Burkitt's Lymphoma in the American population are being studied retrospectively. Techniques include chart review of demographic and clinical data of all patients admitted to the NIH Clinical Center with a confirmed diagnosis of Burkitt's Lymphoma who have primary or metastatic jaw disease.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DE 00332-01 CI

PERIOD COVERED: October 1, 1981- September 30, 1982

TITLE OF PROJECT (80 characters or less): Clinical Investigations and Case Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Roberts, Michael W.	Chief, Patient Care Section	CIPCB	NIDR
Wright, William E.	Sr. Staff Dentist (Periodontics)	CIPCB	NIDR
Drum-Brody, M. Ann	Clinical Staff Dentist	CIPCB	NIDR
Martin, Sue E.	Pathologist	CIPCB	NIDR
Davis, Mary L.	Clinical Associate	MB	NCI
Geffen, David B.	Clinical Associate	MB	NCI
Nelson, Mary J.	Staff Radiologist	ORB	CC
Straus, Stephen E.	Chief of Medical Virology	LCI	NIAIO
Levine, Michael	Clinical Associate	IIGP	CC
Dwyer, Andrew	Staff Radiologist	ORB	CC
Kissane, John M.	Visiting Scientist	LP	NCI
Costa, Jose C.	Chief, Pathological Anatomy Branch	LP	NCI
Chu, Elizabeth W.	Chief, Cytopathology Section	LP	NCI

COOPERATING UNITS (if any): Pathological Anatomy Branch, National Cancer Institute
Inter-Institute Genetics Program, Clinical Center, NIH

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Patient Care Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.1	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS Chart reviews

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical case studies and clinically related research are being conducted on a variety of dentally related subjects. Techniques being utilized include chart and literature reviews and microscopic laboratory application of cell markers as an adjunct to morphological criteria used in cytological diagnosis.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00334-01 CI

PERIOD COVERED: October 1, 1981- September 30, 1982

TITLE OF PROJECT (80 characters or less)

Orthodontic/Periodontal Management of Juvenile Periodontitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Folio, John	Senior Staff Dentist (Orthodontics)	CIPCB	NIDR
Rams, Thomas	Staff Fellow	CIPCB	NIDR

COOPERATING UNITS (if any)

Paul H. Keyes, International Dental Health Foundation, Reston, Virginia

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Patient Care Section

INSTITUTE AND LOCATION: National Institute of Dental Research

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Orthodontic movement in the presence of advanced periodontal disease is rarely indicated. However in adolescents with juvenile periodontitis, function and esthetics may be compromised because of malocclusion. Phase-contrast microscopic monitoring of the subgingival microflora and nonsurgical, antimicrobial therapy were used to minimize periodontal complications commonly found with orthodontic movement of teeth with minimal osseous support in juvenile periodontitis subjects. Both limited and complete edgewise orthodontic movement were coordinated with periodontal therapy stressing maintenance of spirochetes, motile rods and crevicular polymorphonuclear leukocytes at low or undetectable levels. After at least 2 years of follow-up, all orthodontic movement was successful without periodontal complications. This study provides the basis for a wider selection of therapeutic modes of treatment that can be employed on juvenile periodontitis subjects.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00336-01

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Salivary Gland Secretory Mechanisms During Normal and Altered Functional States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Baum, B.J.	Dental Officer	CIPCB, NIDR
Bodner, L.	Visiting Fellow	CIPCB, NIDR
Kousvelari, E.	Expert	CIPCB, NIDR
Roth, G.S.	Research Chemist	CPB, NIA
Uchida, T.	Visiting Fellow	CPB, NIA
Nand, A.	Dental Officer	LBS, NIDR
Qwarnstrom, E.	Visiting Fellow	LBS, NIDR

COOPERATING UNITS (if any)

Endocrinology Section, Clinical Physiology Branch, NIA; Experimental Morphology Section, Laboratory of Biological Structure, NIDR; M.J. Levine and L. Tabak, Department of Oral Biology, SUNY, Buffalo, NY

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.1	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The health status of the oral cavity is in large part influenced by constituents in saliva. The principal function of salivary glands is the production of this complex fluid. To study the mechanisms controlling secretion of salivary components we have utilized both in vitro and in vivo animal preparations. As a probe of secretory events we have primarily focused our studies on peturbations of gland functions that occur with normal aging. In particular the role of specific neurotransmitter induced phenomena to secretory events has been investigated. Included are studies of : (1) protein production, processing and secretion from submandibular gland cells in vitro and its relationship to calcium mobilization; (2) water and electrolyte secretion from the parotid gland (K^+ and Ca^{++} fluxes, α-adrenergic and cholinergic responsiveness) in vitro; (3) parasympathetic control of fluid, electrolyte and exocrine protein release in vivo from parotid and submandibular glands; and (4) adrenergic control of intermediary metabolic functions of the parotid gland during secretion.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00337-01

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Oral Physiological Processes: Normal Function and Disease Perturbation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Baum, B.J.	Dental Officer	CIPCB, NIDR
Bodner, L.	Visiting Fellow	CIPCB, NIDR
Kousvelari, E.	Expert	CIPCB, NIDR
Fox, P.	Dental Officer	CIPCB, NIDR
Cole, M.	Visiting Scientist	NCP, NIDR
Sonies, B.C.	Speech Pathologist	REHAB, CC
Shawker, T.H.	Medical Officer	DR, CC
Costa, P.	Research Psychologist	LBS, NIA

COOPERATING UNITS (if any)

National Caries Program, NIDR; Laboratory of Behavioral Science, NIA; Rehabilitation Medicine and Diagnostic Radiology, Clinical Center, NIH; N.J. Levine, Department of Oral Biology, SUNY, Buffalo, NY

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There bes been little systematic study of the function of tissues within the oral cavity during aging, either describing normal processes or alterations resulting from specific diseases and therapeutic procedures. The purpose of this project is to focus on 3 oral health problem areas for the elderly (salivary secretion, oral motor function and cervical caries) and examine the status of certain biological factors which would likely influence the course of such problems. Major effort has been directed at evaluating electrolyte secretions from the stimulated parotid glands (reflecting ion fluxes in various gland components) and assessing several oral motor functions (postural, masticatory, speech, swallowing).

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00338-01

PERIOD COVERED: October 1, 1982 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Role of Oxygen Radicals in Inflammation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Roffeld, J.T.	Dental Officer	CIPCB, NIDR

COOPERATING UNITS (if any)

None

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies have further defined the role in inflammatory immune responses of endogenous antioxidant mechanisms and their enhancement by the addition of agents such as 2-mercaptoethanol (2-ME) to stimulated cell cultures. After developing a new assay system which permits the measure of extracellular free thiol concentrations in cell cultures, in situ, studies were performed to define the role of thiols as protective antioxidants. We found that cells exposed to various concentrations of mixed disulfides divide and function in proportion to their capacity to cleave those disulfides to free extracellular thiols. This activity required either a mitogenic or antigenic stimulus. Disulfides could be cleaved by T cells, B cells, macrophages or epithelial cells. The antioxidative function of these extracellular thiols was confirmed by observations that high oxygen tension or oxidizing reagents decreased cell proliferation only after and in proportion to decreasing thiol concentrations. Correlative studies indicate that γ-glutamyl transpeptidase is the membrane-bound enzyme responsible for the cleavage of disulfides to thiols.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00339-01

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less): Regulation of Osteogenic Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Somerman, M.	Staff Fellow	CIPCB, NIDR
Termine, J.D.	Research Chemist	LBS, NIDR
Reddi, A.H.	Research Biologist	LBS, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: CIPCB, NIDR, NIH

SECTION: Clinical Investigations

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Extracts of bone tissues are being investigated in order to identify bone specific proteins and to characterize their structure and biological function. A heat labile, trypsin sensitive protein (M_r 65,000) with chemotactic activity for 'osteoblast-like' cells has been identified and partially purified from guanidine extracts of demineralized rat bone matrix powder and from guanidine EDTA extracts of both embryonic human bone and rat calvaria. Future studies will include the examination of cementum proteins and the isolation of cementoblasts in order to understand the mechanisms regulating periodontal ligament-cementum attachment interactions. Studies will also continue on the purification and biological function of the bone chemotactic protein.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00340-01

PERIOD COVERED: October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less): Role of Cytophaga species in Periodontal Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Fox, P.C.	Dental Officer	CIPCB, NIDR
Donahue, A.N.	Staff Fellow	CIPCB, NIDR
Somerman, N.	Staff Fellow	CIPCB, NIDR
Siraganian, R.P.	Medical Officer	LMI, NIDR
Kagermeier, A.S.	Visiting Fellow	LMI, NIDR
Kolenbrander, P.	Staff Fellow	LMI, NIDR
London, J.	Research Scientist	LMI, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines the role(s) of the gram negative oral microorganism Cytophaga sp. in the initiation, development and progression of periodontal diseases. These bacteria are constituents of dental plaque and are strongly implicated in the pathogenesis of destructive periodontitis. A series of monoclonal antibodies have been developed, utilizing both rat and mouse, with specificity for human oral isolates of Cytophaga sp. and the related species, Capnocytophaga. These reagents have been used to demonstrate the heterogeneity of these species as they exist in the oral cavity. These antibodies are useful as well in continuing studies of attachment and aggregation of these bacteria. Attachment of bacteria to the tooth surface or aggregation to previously attached bacteria represents the initial step in the periodontal disease process. An in vitro attachment assay has been developed for Cytophaga sp. bacteria to examine mechanisms of attachment and the role of serum and serum-derived factors in the attachment process.

PHS-6040 (Rev. 2-81)

DIAGNOSTIC SYSTEMS BRANCH

The shift in research emphasis of DSB toward systematic analysis of factors influencing diagnosis of oral and related disorders mentioned last year has been intensified at the expense of some anatomically based research involving functional description of the oropharyngeal complex. The rationale for this shift reflects both changing research priorities within the Branch, and a winding down of research commitments within the Oral and Pharyngeal Development Section pending retirement of that Section's Chief, Dr. James T. Bosma effective the end of FY 82. By this time it is anticipated that all OPD personnel will have been relocated permanently, and the Section will cease to exist.

The Diagnostic Methodology Section has been actively pursuing theoretical analysis of method-specific diagnostic processes with recent emphasis on *in vitro* analyses of clinically promising x-ray systems. To this end DMS investigators have been working closely with scientists of the X-ray Physics Group, NBS, via an NIDR interagency agreement to develop a prototype x-ray system which permits accurate reproduction of exposure geometry from one examination to the next. This work also has fostered DMS participation in the development of a portable dental fluoroscope, an effort being funded by the US Army Institute of Dental Research by DMS scientists in association with the Astrophysics group at NASA in previous years.

Of particular interest is work relating x-ray projection angles to statistically determined limits of contrast detectability. The underlying model assumes a variable correlation between specific image elements which determine lesion detectability. This work extends previous efforts underlying the determination of factors limiting diagnostic performance obtainable from capacity-limited systems, and provides a quantitative basis for incorporating biological variation into the analysis.

Other work has shown that computerized tomographic reconstruction of dental tissues can result in clinically interesting 'slices' when generated from as few as nine discrete projections having angular disparities from normal of not more than four degrees. This finding is of practical significance because the same hardware being developed for stabilizing projection geometry described above, may find application in computerized dental tomography with little if any modification.

Although DMS has not emphasized research underlying the application of symmetric-axis geometry to the description of the mandible because of the shift of priorities described last year, significant progress has been made thanks to collaborative efforts initiated by investigators at Tufts University who were stimulated to continue this work after becoming familiar with our previous efforts in this area.

This work confirms previous conclusions regarding the stability of symmetric-axis segment ratios. It shows also that portions of the chin grow at a rate which is significantly different from that associated with longitudinal lengthening of the corpus and ramus. This finding is consistent with data published earlier by DMS investigators involving the use of symmetric-axis descriptors to facilitate studies of differential growth of the mandible. Related data dealing with angular stability of the jaw are still waiting to be analyzed statistically by DCRT scientists.

Plans for the future include the recruitment of Dr. Hans Grondahl to the DSN research team. Dr. Grondahl, a former visiting scientist from Gothenburg, Sweden, is an expert in the clinical application of radiological resources in dentistry, and is expected to play a prominent role in the planned development of a clinical research program involving the application of new and improved diagnostic methods in dentistry.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00048-10 DS

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Anatomical Studies of the Head

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Bosma, J.F. Chief, Oral Pharyn Dev Sec NIDR DS

COOPERATING UNITS (if any)
Division of Research Services, MAPE

LAB/BRANCH
Diagnostic Systems Branch, NIDR

SECTION
Oral and Pharyngeal Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.35	.00

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The book, Anatomy of the Infant Head, is essentially ready for publication.

The Application for publication subvention, which was submitted by The Johns Hopkins University Press to the National Library of Medicine, NIH, was approved, but limitation of NLM funds prevents this sponsorship.

Publication subvention sponsorship is now being sought from a private foundation.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00065-11 DS

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Development of Evaluation of Improved Dental Radiographic Systems with Emphasis on Factors Influencing Diagnostic Performance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Webber, R.L.	Dental Director	NIDR	DS
Ruttimann, U.E.	Sr. Staff Fellow	NIDR	DS
Reese, J.A.	Hlth Scntst Admr	NIDR	RMP

COOPERATING UNITS (if any)
National Bureau of Standards, X-ray Physics Group

LAB/BRANCH
Diagnostic Systems Branch

SECTION
Diagnostic Methodology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.96	1.33	1.63

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The effect of spatial and temporal correlations between specific image elements which determine the detectability of small changes in radiopacity in tissue of diagnostic interest are being evaluated in vitro using computer simulations and quantitative measurements derived from radiographic phantoms.
This work compliments collaborative research done in association with extramural programs staff and the Restorative Materials Program Branch, and the National Bureau of Standards to develop a prototype x-ray system which permits accurate reproduction of exposure geometry from one examination to the next. The system will contain a quantum-efficient, non-film, intraoral detector coupled with some sort of scanning x-ray source which produces computer-processed digital images in near real time.
Other studies consider the applicability of information theory to the description of diagnostic performance obtainable from radiographic systems, and the use of computerized tomosynthesis to describe dental structures using limited-angle projection geometry.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00158-08 DS

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cephalometric Description of Growth Processes Through the Use of Symmetric Axis Coding

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Webber, R.L.	Dental Director	NIDR	DS
Hosimann, J.E.	Chief	DCRT	LSM

COOPERATING UNITS (if any)
Laboratory of Statistics and Mathematical Methodology, DCRT
Tufts University, School of Dental Medicine, Department of Oral Pediatrics

LAB/BRANCH
Diagnostic Systems Branch

SECTION
Diagnostic Methodology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.10	.65

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Analysis of bone growth and development using symmetric-axis geometry is being applied to digitized cephalometric tracings obtained from an existing data base at the University of Michigan. Specifically studied are lateral projections of the mandible produced from normal children at various ages. Previous findings indicated that symmetric-axis angles determined at points of segmental intersection were relatively constant within and between individuals irrespective of age. More recent work done in collaboration with investigators at Tufts University demonstrate relative constancy in symmetric-axis segment ratios measured within individuals. The data show that the proportions remain relatively stable irrespective of initial segment length and age. These results show that a potential exists for using patients as their own controls when analyzing segment development.
Future plans will continue to rely heavily on the ability to maintain collaborative associations with other investigators concerned more directly with morphometrics.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00181-05 DS

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Postnatal Development of the Rat Skull

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Bosma, J. F. Chief, Oral Pharyn Dev Sec NIDR DS

COOPERATING UNITS (if any)
National Library of Medicine
Philadelphia Children's Hospital
University of Michigan

LAB/BRANCH
Diagnostic Systems Branch

SECTION
Oral and Pharyngeal Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.25	.20

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A book, Postnatal Development of the Rat Skull, authored by Melvyn J. Baer, Ph.D., James F. Bosma, M.D., and James L. Ackerman, D.M.D. is now in stage of page proofs at the University of Michigan Press, Ann Arbor. The book should be off the press in October of this year.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00182-05 DS

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of Sensorimotor Impairments of the Mouth and Pharynx

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Bosma, J.F.	Chief, Oral Pharyn Dev Sec	NIDR DS

COOPERATING UNITS (if any)
Johns Hopkins Medical Center; CC; NICHD; NINCDS.

LAB/BRANCH
Diagnostic Systems Branch

SECTION
Oral and Pharyngeal Development Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.40	.00

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Anatomical abnormalities and performance impairments of the oro-pharyngeal complex are evaluated clinically in patients suffering from a variety of disorders. The primary goal is to characterize dysphagia in terms of specific cineradiographic patterns which can be used to distinguish various types of functional deficit. Recent findings demonstrate significant changes in the sequence of events precipated by voluntarily-elicited pharyngeal swallow depending on the type of dysphagia encountered. These changes often lead to volunatrly-mediated compensatory mechanisms which impact on feeding patterns and dietary preferences.

The clinical significance of this project is reflected in the establishment of a Swallowing Center at the Johns Hopkins Medical Center which is directed toward the clinical study, diagnosis and treatment of patients suffering from dysphagia with particular emphasis on problems secondary to neurological impairment.

A book, Radiography of the Pharynx, is in preparation.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00211-06 DS

PERIOD COVERED
October 1, 1981 to September 30, 1982.

TITLE OF PROJECT (80 characters or less)
Enhancement of Diagnostic Images

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Webber, R.L.	Dental Director	NIDR	DS
Ruttimann, U.E.	Sr. Staff Fellow	NIDR	DS
Groenhuis, R.	Visiting Fellow	NIDR	DS

COOPERATING UNITS (if any)

LAB/BRANCH
Diagnostic Systems Branch

SECTION
Diagnostic Methodology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.23	1.49	.74

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This project is an extension of previous work involving the creation, development and testing of image-processing techniques designed to improve diagnostic performance.
New algorithms for eliminating the effects of artifacts in subtraction radiographs have been devised. They involve position-invariant contrast manipulation, and contast-dependent analysis of grey-level changes produced from sequentially-obtained dental radiographs.
Other work involves the use of image-processing techniques to enhance radiographic images produced digitally for use in tomosyntheais. One approach explores the use of spatial-frequency filtering techniques on x-ray projections prior to tomographic reconstruction. Another is based on an iteration scheme which eliminates artifacts produced by blurring of structures projected sharply in adjacent planes.
Preliminary data suggest that these algorithms can eliminate artifacts which significantly compromise diagnostic performance obtainable from subtraction radiographs and tomosynthetic reconstructions.

PHS-6040 (Rev. 2-81)

NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

The Neurobiology and Anesthesiology Branch is concerned with the study of oral-facial sensation, with particular emphasis on mechanisms of pain and the development of new methods for controlling pain in humans. The Branch is composed of three sections that utilize anatomical, physiological, behavioral, pharmacological and psychophysical techniques to study neural function as it relates to the processing of sensory signals about the threat of tissue-damaging stimulation. The Neural Mechanisms Section includes the following research activities: 1) correlative morphological, physiological and neurochemical studies of the organization of the medullary and spinal dorsal horns and the identification of putative neurotransmitters involved in sensory transmission; 2) correlative behavioral and physiological studies to determine the role of different peripheral and central neural populations in pain and temperature discrimination. The Neurocytology and Experimental Anatomy Section is concerned primarily with the study of synaptic connections in the medullary and spinal dorsal horns in normal tissue and following peripheral nerve injury. The Clinical Pain Section develops new methods for measuring and assessing experimental and clinical pain and applies these methods to the study of various pharmacological and non-pharmacological techniques potentially useful in the control of anxiety, apprehension and pain associated with dental procedures and in the treatment of chronic pain.

This year we have continued our detailed analyses of the organization of the medullary and spinal dorsal horns and their role in pain transmission. By combining techniques from different disciplines we have been able to elucidate functional circuits within the dorsal horn that play a role in information transfer related to pain, temperature and touch sensation. Recent studies of these systems following peripheral nerve injury are beginning to shed light on the morphological basis of some chronic pain states precipitated by the loss of sensory input. Our animal research studies also provide the conceptual framework for human studies on mechanisms of acute postsurgical pain and chronic pain conditions. Human studies also have focused on improved methods for assessing acute and chronic pain and the evaluation of new analgesic and anti-anxiety agents useful in controlling postsurgical pain.

We have continued to develop our clinical pain research efforts by increasing our collaboration with other Institutes. Studies on pain associated with diabetic neuropathies, oral-facial pain, low back pain and cancer pain are in progress. Present plans are to relocate the clinical pain program to the Clinical Center Ambulatory Care Research Facility in the fall of 1982. The Clinical Pain Section will coordinate this multi-Institute collaborative program on clinical pain research.

Investigators in the Branch received recognition for their achievements by being chosen to chair and present their research at symposia and workshops at the annual meetings of the Society for Neuroscience and the International Association for Dental Research. In addition, Drs. Dubner and Gobel were honored by their election to Vice-President, International Association for the Study of Pain and to President, Neuroscience Group, International Association for Dental Research, respectively.

The Neural Circuitry of the Medullary and Spinal Dorsal Horns

The lower end of the spinal trigeminal nucleus in the brain stem, called trigeminal nucleus caudalis, is directly continuous with the spinal dorsal horn and is homologous to it in terms of structure, chemistry and physiological function. For these reasons, it is more properly referred to as the medullary dorsal horn. This year we have continued our in-depth studies of the functional organization of the medullary and spinal dorsal horns and their role in pain mechanisms.

The synaptic circuitry of the dorsal horn consists of three major components: 1) the central terminals of primary afferent nerve fibers whose peripheral receptive terminals innervate the skin, muscles and viscera; 2) intrinsic neurons whose cell bodies lie in the dorsal horn; and 3) the central terminals of supraspinal and brain stem neurons that modify the output of the intrinsic neuronal dorsal horn system. The intrinsic system contains two major types of neurons: those whose processes form local neuronal circuits within the dorsal horn and those that send their projections out of the dorsal horn to other central nervous system structures. This year we have continued to examine the properties of local circuit neurons in the superficial layers and, in addition, have studied major projection neuron systems in the dorsal horn. Laminae I and II contain identified neuronal cell types that respond exclusively to noxious stimuli (nociceptive-specific), respond to both innocuous and noxious stimuli (wide-dynamic-range) or respond only to innocuous stimuli (located in layer IIb only). These superficial layers also contain a wealth of chemical mediators that are released by primary afferent or descending neurons projecting to this region as well as by intrinsic neurons. Using immunocytochemical techniques alone or in combination with the retrograde and intracellular horseradish peroxidase (HRP) methods, at both light and electron microscopic levels, we are examining the

role of these putative neurotransmitters in the neural circuitry of the superficial dorsal horn.

We previously demonstrated that the superficial layers contain enkephalinergic neurons that have light- and electron-microscopic characteristics of lamina IIb islet cells, a local circuit neuron originally identified in Golgi and intracellular HRP studies. Recent studies have demonstrated a second enkephalinergic neuron that has the morphological characteristics of lamina IIa stalked cells, another local circuit neuron. Since not all stalked and islet cells were labelled with enkephalin antisera, it appears that these local circuit neurons or interneurons may be neurochemically diverse and possibly participate in different functional circuits. For example, some stalked cells may be excitatory interneurons relaying input to lamina I projection neurons, whereas others, possibly containing enkephalin, may be inhibitory interneurons participating in segmental and descending modulatory effects.

During the past year, the major neuronal cell types in the superficial layers of the dorsal horn were studied at the ultrastructural level after being labelled with the intracellular HRP method. Only the laminae IIa and IIb islet cells contained synaptic vesicles in their dendrites and participated in dendrodendritic synapses. These dendrites thereby function as sources as well as receivers of synaptic inputs in laminae I and II. All cell types studied in these layers received input from dome-shaped endings that originate in part from descending axons of aminergic neurons in the brain stem. The lamina I or marginal neurons and the stalked cells in lamina II received the most extensive input from dome-shaped axonal endings, whereas the islet cells of laminae IIa and IIb contained the fewest number. These findings are consistent with our interpretation that these morphologically distinct neuronal cell types subserve different functional roles in the transmission of somatosensory information. Marginal neurons and stalked cells appear to directly or indirectly transmit peripheral input to more rostral central nervous system sites whereas islet cells modulate this transfer of information via local dorsal horn circuitry. Descending effects appear to influence this rostral transfer of information mainly via postsynaptic actions on marginal and stalked cells.

Studies of projection neurons in the deeper layers of the dorsal horn using the retrograde HRP method have revealed the presence of a major system reaching the brain via the dorsal columns. This dorsal column postsynaptic (DCPS) system contains about 1000 neurons in the lumbosacral enlargement of cats and monkeys and appears to be one of the major sources of somatosensory input from the spinal cord to higher brain centers. These neurons are concentrated mainly in laminae III and IV. Electrophysiological studies have shown that approximately one-half of DCPS neurons respond only to innocuous tactile stimuli while the remainder respond to innocuous stimuli but exhibit a higher frequency discharge to noxious stimuli. Several DCPS neurons were successfully impaled and injected with HRP to study their complete morphology. Nearly all of the neurons had dendritic arbors that were elongated in the longitudinal axis of the spinal cord but relatively narrow in the transverse axis. Four morphological types of DCPS neurons have been identified. DCPS neurons rarely sent their dendrites into laminae I and II and the particular morphology of a cell could not be correlated with its physiology. Many DCPS neurons issued axon collaterals that arborized at the level of their cell body.

Major advances have been made this year in defining the interaction of neurochemically defined intrinsic and descending pathways with projection neurons in the dorsal horn. By combining two powerful techniques, the HRP method and immunocytochemistry, we have been able to label two components of dorsal horn circuitry in the same experiment. Using these techniques, recent studies have made the first anatomical demonstration of a synaptic relationship between axonal endings containing an opioid peptide, enkephalin, and an identified postsynaptic neural process in the dorsal horn. Enkephalin immunoreactive axonal endings were shown to make direct synaptic contact with the somata and proximal dendrites of laminae I and V trigeminothalamic and spinothalamic neurons of cat and monkey retrogradely labelled with HRP. These observations are important since they demonstrate that opiates modulate the transfer of nociceptive information in the dorsal horn via postsynaptic mechanisms acting directly on projection neurons.

The presence of serotonin, a putative neurotransmitter involved in the descending modulation of nociception, also was investigated. Serotonin immunoreactive contacts were observed on the somata and proximal dendrites of thalamic projection neurons in laminae I and V. Thus, descending brain stem monaminergic modulation of nociceptive input also involves postsynaptic receptors located on projection neurons. In similar studies, DCPS neurons retrogradely labelled with HRP received direct contacts from serotonin immunoreactive axonal varicosities on their somata and proximal dendrites. Such findings imply that descending serotonin systems modulate physiologically diverse types of neurons since some DCPS neurons respond exclusively to tactile input while others are nociceptive neurons.

A critical role of serotonin in modulating the output of dorsal horn interneurons was demonstrated in other

studies. Ultrastructural observations revealed that serotonin immunoreactive axons were found in all laminae although their numbers were greatest in laminae I and IIa. Labelled endings were primarily dome-shaped and formed a single synapse, most commonly on small caliber dendritic shafts. The endings contained either pleomorphic or round granular vesicles and a few dense core vesicles. These observations provide additional evidence that serotonin exerts its modulatory effects on non-nociceptive and nociceptive neurons via postsynaptic mechanisms rather than presynaptic effects on incoming primary afferent axons. Similar dome-shaped endings have also been found to contain noradrenalin, another putative neurotransmitter originating from cells in the brain stem. These noradrenergic dome-shaped endings, like their serotoninergic counterparts, synapse mainly on small caliber dendrites. A few scalloped-shaped noradrenergic endings also have been found in laminae I and IIa.

By combining immunocytochemical techniques with the intracellular HRP method, we have been able to examine, at the light microscope level, the distribution of serotonin contacts on morphologically and functionally identified neurons in the superficial dorsal horn. Serotonin immunoreactive contacts were found on marginal neurons in lamina I and stalked and islet cells in lamina II. For all three neuron types, both nociceptivespecific and wide-dynamic-range neurons were represented. For all three cell types, serotonin immunoreactive axonal contacts occurred preferentially on dendritic shafts rather than on spines. The number of serotonin contacts on marginal and stalked cells was much greater than on islet cells. Axonal contacts were concentrated in the proximal 250 m of the dendritic tree of marginal and stalked cells, but were more evenly distributed in the dendritic trees of islet cells. Stimulation of nucleus raphe magnus in the brain stem, a major site of origin of serotonin input, consistently resulted in inhibition of the nociceptive responses of marginal and stalked cells. Similar stimulation failed to influence the activity of islet cells. These findings confirm our previous interpretation that morphologically distinct cell types subserve different roles in dorsal horn function and that serotonin descending modulation is exerted via postsynaptic mechanisms mainly on neurons concerned with the rostral transfer of nociceptive information (marginal and stalked cells).

The experiments described above have identified several important sites of action of neurotransmitters in the dorsal horn. The analysis of monoaminergic axonal endings is of particular significance since the activation of descending aminergic pathways are implicated in mechanisms of analgesia. The study of enkephalinergic neural circuitry furthers our understanding of the role of opiates in pain and other somatosensory pathways.

Another important question relating to neural circuitry in the dorsal horn concerns the identification of the termination sites of different types of primary neurons activated by innocuous and noxious stimuli. Using immunocytochemistry combined with the intracellular HRP method, we have examined the distribution of substance P contacts on identified superficial dorsal horn neurons. Substance P is a candidate neurotransmitter for small primary afferents activated by nociceptive input. Although substance P also is found in intrinsic dorsal horn neurons (and possibly descending axons), these studies do give some insight as to the possible termination sites of substance P primary axons. In contrast to serotonin contacts, substance P contacts preferentially occurred on spine heads rather than on dendritic shafts, although aspiny neurons had substance P contacts on dendritic shafts.

What happens to primary afferent neurons after peripheral nerve injury? In studies begun this year, the effects of such deafferentiation on dorsal horn synaptic circuitry was examined in detail. A number of findings indicate that peripheral nerves separated from their cutaneous receptive zones remain in place for up to 90 days. First, primary cell bodies of all sizes survived the injury. Second, the central axonal arbors of these injured primary neurons remained intact. Third, these endings maintained their synaptic vesicles and some of their synaptic connections in the dorsal horn. In spite of the maintenance of these primary afferent fibers, ultrastructural studies revealed that many dendrites of neurons in laminae I-III showed changes as a consequence of the loss of primary afferent input. There appears to be a loss of dendrites via the formation of large dendritic cavities and eventual fusion of membranes and opening to the intercellular space. This loss of dendrites in spinal dorsal horn parallels changes previously seen in medullary dorsal horn neurons following the loss of tooth pulp primary inputs. It will be important to determine whether such changes in dorsal horn circuitry provide the morphological substrate for patho physiological mechanisms associated with some chronic pain states.

Behavioral Correlates of Neural Function in the Medullary Dorsal Horn

We have extended our analysis of the neuronal properties of the medullary dorsal horn by correlating response characteristics with behavior in awake monkeys trained in sensory discrimination tasks. As mentioned above, two general classes of dorsal horn neurons (widedynamic-range and nociceptive-specific), studied in anesthetized animals, convey information

related to pain. Our major objectives this year were 1) to continue studies of medullary dorsal horn neurons that send axonal projections to the thalamus and 2) to determine discrimination levels to thermal stimuli in humans and monkeys utilizing a newly developed behavioral task. The new thermal discrimination task requires that subjects report which of two simultaneously-applied heat pulses is warmer. Two contact thermodes are positioned symmetrically on the subject's face. The baseline temperature of the probes is 35°C. At the beginning of each trial a panel is illuminated. The subject presses the illuminated panel and is presented simultaneous heat pulses, one on each probe. The risetimes of the heat pulses are identical, but the final temperatures differ. The subject presses the left panel if the thermode on the left side of the face is warmer or the right panel if the right thermode is warmer. In some sessions the subject compares a 47°C stimulus to less intense stimuli while in other sessions he compares a 39°C stimulus to less intense stimuli. This task provides a psychophysical measure to determine difference limens for thermal intensities in the innocuous and noxious ranges. All subjects (1 monkey and 4 humans) showed a positive relationship between correct responses and the magnitude of the temperature difference. In addition, for temperature differences greater than 0.1°C, all subjects produced more accurate discriminations in the noxious range (47°C) than in the innocuous range (39°C). The difference threshold, defined as the smallest temperature change detected on 75% of the trials, was smaller for every subject at 47°C than at 39°C. This increased discriminative ability in the noxious heat range suggests that there is more secure central nervous system processing of stimulus intensity information arising from thermal nociceptors than from warm fibers. Such a task can be utilized to correlate neural activity in central nervous system pain pathways in monkey with behavioral discrimination. It permits an unequivocal demonstration of those neurons necessary or sufficient for the discrimination of painful stimuli.

Related studies utilizing a similar behavioral task examine the effect of attention on the subjects' ability to detect the onset of thermal stimuli. The subject is aided in making this detection by the presence of a warning light above the correct response panel. Reaction times for detecting the temperature change and accuracy in responding are used to determine the effects of warning signals on subject performance. This task will allow us to evaluate the effect of attentional variables on behavioral performance and neuronal activity in monkeys. Such findings should be important in determining those brain pathways involved in the non-pharmacological modulation of pain signals.

In detection tasks described previously, monkeys are trained to detect the termination of innocuous thermal stimuli (37° - 43°C) and the onset of noxious heat stimuli (45° - 49°C) applied to the face (thermal task). In a visual task, the same monkeys detect the onset of a visual stimulus while behaviorally irrelevant thermal stimuli are presented. Neuronal activity in the medullary dorsal horn is correlated with a number of behavioral events such as panel press, temperature onset, temperature termination, panel release and reinforcement delivery. Our most recent studies have confirmed and extended findings previously reported for nonprojection trigeminal neurons recorded in anesthetized and unanesthetized monkeys. We have found that in awake monkeys, two types of neurons that project to the thalamus are responsive to noxious thermal stimuli: wide-dynamic-range (WDR) neurons, which respond differentially to innocuous and noxious mechanical stimuli, and nociceptivespecific (NS) neurons, which respond exclusively to intense or noxious stimuli. Thermal response thresholds range from 41° to 47°C, and stimulus response functions are monotonic from threshold to 49°C. For both WDR and NS trigeminothalamic neurons, greater neuronal discharges are associated with shorter behavioral discrimination latencies. These data show that thermally sensitive WDR and NS neurons transmit information to the thalamus that correlates with the monkey's ability to discriminate noxious thermal stimuli. Therefore, these neurons appear to participate in neural mechanisms underlying the sensory-discriminative aspects of pain.

We also found that the thermal sensitivity of trigeminothalamic neurons was influenced by the behavioral relevance of the thermal stimuli. For some neurons the neuronal discharges were greater when thermal stimuli were presented in the thermal task (and necessary for reward) than when they were presented in the visual task (and not necessary for obtaining reward). These data indicate that behavioral relevance is a critical variable influencing the response magnitude of sensory-discriminative neurons and provide evidence that cognitive factors can modulate the output of sensory neurons at a very early stage of central nervous system processing.

Previously, we have described responses of thermosensitive and mechanosensitive medullary dorsal horn neurons that are independent of stimulus modality or stimulus parameters. In the present project we investigated these task-related properties in more detail. We identified several types of task-related responses. Some cells discharged when the monkey initiated the trial. Others discharged at the signal for panel release, whether that signal was a temperature change or light onset. The most common pattern of

task-related activity was a transient or sustained discharge at trial initiation and an additional burst discharge after the signal for panel release. Task-related responses occurred only during performance of a task and were related to sensory events that led to successful completion of the task. Such responses were not correlated with specific face, arm or hand movements. Some neurons with each pattern of task-related activity projected to the thalamus. Neurons with task-related activity may be providing a gain control mechanism for somatosensory information that the animal must use for successful completion of the task. Additionally, these responses may be involved in the transmission of behavioral information to motor cortex to facilitate appropriate goal-directed behaviors.

These studies are important in determining the neurons critical for signalling the intensity of painful thermal stimuli and transmitting this information to levels of conscious sensation. By studying the monkey's behavioral responses within a task we also can assess the influence upon pain perception of such variables as behavioral significance and predictability of noxious thermal stimuli. Concurrently, we can study modulation in activity of neurons involved in the transmission of noxious information from the face. Our data show that the neural representation of oral-facial nociception can be influenced by environmental and behavioral factors at the earliest stage of central integration and that this modulatory information is relayed to a thalamic nucleus that receives thermal sensory information. This work is a behavioral demonstration of non-pharmacological modulation of neurons involved in oral-facial nociception, and consequently is important in understanding non-pharmacological approaches to the control of oral-facial pain.

The Assessment of Experimental and Acute Clinical Pain

The purpose of these human studies is 1) to develop psychophysical and behavioral models of pain perception that assess the intensity and unpleasantness of experimental and clinical pain sensation and also assess the ability of subjects to judge their perceptual experience, and 2) to use these models to assess physiological and psychological mechanisms of pain and analgesia, and the efficacy of pharmacological and non pharmacological methods of pain control.

We are continuing to study mechanisms of postoperative dental pain following the extraction of impacted third molars. Pain is assessed for one hour before and after intravenous injection of fentanyl, saline, or naloxone, or after no treatment, by visual analog and verbal descriptor scales of sensory intensity, unpleasantness and painfulness. The results showed that naloxone increases, and saline placebo decreases, postsurgical pain by separate and independent mechanisms. These results indicate that placebo analgesia, which occurred in either the presence or absence of endogenous opioid like compounds, is mediated by nonopioid mechanisms. Naloxone increased postsurgical pain independent of the placebo effect, implicating antagonism of endogenous opioid like compounds released as a consequence of surgical stress. Previous studies of dental postoperative pain suggest that naloxone reverses placeboproduced analgesia. The present postsurgical pain study addressed this issue with a refined design that permitted the separate assessment of the effect of naloxone and placebo. The results show that naloxone and placebo mechanisms are separate and independent. These results are sufficient to explain previous results found after naloxone and placebo administration.

An additional study assessed the relationship between the reduction in the magnitude of verbal pain reports following analgesic administration and the reduction in actual sensory experience. Patients used either a numerical category or verbal descriptor scale to rate the intensity of painful thermal stimuli applied to their forearm. After a placebo medication, the intensity of these stimuli were then reduced by a fixed amount on one-half of the occasions to simulate pain reduction after an analgesic. Preliminary results show that patients' verbal reports accurately reflect the level of stimulation. The reduction in responses after the "sham" analgesic corresponded exactly to the amount of stimulus reduction. The results of this study provide strong evidence for the validity of these measures in analgesic assessment.

Another study investigated the types of sensations evoked by tooth pulp stimulation. The tooth has been assumed to be an exclusive source of pain and therefore a unique model for the study of pain, pain pathways and new pain control agents. This study evaluated the characteristics of non-pain and pain sensations evoked by electrical stimulation of the tooth pulp in humans. Detection threshold, defined as the first sensation perceived, and pain threshold were determined and the magnitude of sensations between these thresholds was scaled with verbal descriptor methods and magnitude estimation procedures. Detection thresholds were stable over experimental sessions and independent of the frequency of the stimulating current. Pain threshold, on the other hand, varied as a function of frequency with a minimum value at 100 Hz. Stimuli that evoked non-pain sensations at low frequencies evoked pain sensations when frequency was increased from 5 to 100 Hz. Subjects were able to scale non-pain sensations over a range of

stimulus intensities and frequencies. The lowest currents evoked sensations that were nonpainful and were of constant magnitude despite changes in the frequency of stimulation. Higher stimulus current evoked sensations that were non-painful at low stimulus frequencies and painful at high stimulus frequencies. These findings suggest that the lowest threshold non-pain sensations evoked in tooth pulp are mediated by a distinct population of afferents not involved in the coding of pain. High frequency stimulation of the lowest threshold pulpal afferents results in no summation of nonpain sensation and never produces pain. However, high frequency stimulation evokes greater magnitude sensations at higher stimulus currents, indicating that central summation mechanisms are critical for higher threshold afferents signalling more intense sensations.

Recent studies on pain measurement assessed the contributions of word meanings and category sequences to the perceptual values assigned by subjects in reporting the magnitude of skin stimuli. The results showed that category judgments were made on the basis of word meaning rather than position on a list and that the assumption of equal spacing between verbal categories on a list may be incorrect. These findings provide additional evidence that verbal descriptor scaling procedures produce more information about the perception of stimuli than do simple numerical estimation procedures and should be employed in the assessment of new analgesic agents.

Assessment of Chronic Pain

We are continuing to evaluate the effects of narcotic analgesics and electrical brain stimulation on clinical and experimental pain in a group of chronic pain patients, some of whom received chronic brain electrode implants for pain relief. These electrodes are placed in brain pathways where they are presumed to activate descending, opiate related, pain-suppressing systems. Fifteen patients were assessed during this year. Twelve were admitted for a preliminary screening evaluation. Of these, 8 were unable to complete our assessment procedures and were rejected from further study. Three underwent a full screening evaluation, which included assessing the effects of narcotic agonists and antagonists on the magnitude of their clinical pain and on their responses to noxious thermal stimulation. Deep brain electrodes were implanted in two of these patients and its effects were assessed in both immediate and follow-up postoperative evaluations. Deep brain stimulation produced satisfactory pain relief in both patients. However, the time course of the stimulation-produced analgesia exceeded that of opiates such as morphine, and the magnitude of the analgesic effect was no greater than that observed after sham stimulation. In addition, this analgesia was not reversed by the narcotic antagonist, naloxone. Two patients who received electrode implants last year were also admitted twice each for long term follow-up evaluations. Both had received excellent pain relief from stimulation immediately after implantation, but both complained that stimulation was no longer effective. Testing confirmed that stimulation was not reducing the magnitude of clinical pain or the magnitude of pain produced by noxious thermal stimulation.

The use of deep brain stimulation to control human pain evolved from the findings in animals that electrical stimulation of peri-aqueductal sites activated a descending analgesic system mediated by endogenous opioid like compounds. The human brain stimulation procedure is assumed also to activate a descending opioid system. The present findings that the stimulation-produced analgesia does not show an opioid time course and is not reversed by a narcotic antagonist suggest that the analgesia is not produced by an opioid mechanism. The reduced analgesia found after one year questions the efficacy and ultimate clinical utility of this procedure.

An additional study assessed the effect of morphine, naloxone and placebo on thermal sensations mediated by A-delta and C fiber primary afferent fibers. Patients pressed a button to indicate when each of a series of 51°C stimuli became painful. Responses to the first two stimuli in a series reflect activation of A-delta fibers and responses to later stimuli reflect activation of C fibers. Morphine, in comparison to either placebo or naloxone, increased the latency of A-delta responses and also decreased the number of C fiber stimuli described as painful. These results suggest that morphine exerts differential effects on Adelta and C fiber mediated pain. This method may be one of the first procedures capable of assessing the effect of pharmacological and nonpharmacological analgesic manipulations on separate primary afferent systems.

Another study assessed the effect of stimulus range on the scaling of thermal stimuli. In a previous study we showed a significant reduction of verbal descriptor responses but not handgrip responses after morphine administration in comparison to placebo. This lack of effect with handgrip responses may represent a motoric effect (the subject was unable to physically use a hand dynanometer after morphine administration), an attentional effect (the subject simply stopped attending to the task after drug administration and thus did not respond) or a range effect (subjects were not discriminating well due to the range of stimuli used). In this study, 50 subjects rated thermal stimuli before and after the double-blind administration of fentanyl or saline placebo. One group determined their own

tolerable pain range (45.6° - 48.4°C) prior to testing. This group then received stimuli within their pain range and responded both by squeezing a hand dynomometer and by choosing a verbal descriptor of sensory intensity to describe the magnitude of their pain. A control group did not determine their own pain range and received stimuli within the 45° - 51°C range and responded in the same manner as the first group. A significant effect was seen for the verbal descriptor response for both groups. A significant effect was also found for the handgrip response in the group that determined its own pain range. These results suggest that handgrip responses are sensitive to a drug effect when the pain range is determined by the subject prior to testing. Subjects in the control group could not use the hand dynamometer effectively presumably because many of the more intense stimuli were at the maximal level of tolerance, resulting in a "ceiling effect."

Control of Pain and Anxiety in Ambulatory Dental Patients

These investigations are evaluating novel drugs for controlling postoperative pain in an attempt to identify agents which possess greater analgesic efficacy or less side effect liability than standard agents. Standard therapy with postoperative analgesics usually involves the administration of a narcotic analgesic in combination with a mild analgesic, such as aspirin or acetaminophen. The use of narcotics in ambulatory patients is associated with nausea, vomiting and dizziness. The drugs under investigation in our studies are selected on the basis of having greater efficacy or lower side effect potential.

A within-subject, double-blind crossover design is being employed in these investigations. Patients in need of bilateral extraction of impacted third molars serve as subjects. Subjects receive one of the two treatments on a random basis at the first appointment and the alternative treatment is administered at a second appointment, approximately two weeks later. Flurbiprofen, a non-steroidal anti-inflammatory agent, in combination with etidocaine, a long-lasting local anesthetic, was compared to standard treatment. Following the extractions, subjects remained at the clinic to rate their postoperative pain. The combination of flurbiprofen and etidocaine resulted in less postoperative pain then standard treatment with oxycodone plus acetaminophen and lidocaine. Approximately one-third of the patients in the sample reported no postoperative pain during the seven hour observation period at the clinic following the experimental combination. Significantly fewer patients reported side effects following the flurbiprofen plus etidocaine treatment, indicating that the enhanced clinical efficacy of the combination was not at the expense of an increased side effect liability. These findings indicate that the combination of a non-steroidal anti-inflammatory agent and a long-acting local anesthetic provides superior postoperative pain relief than analgesic methods presently employed. The increase efficacy of these agents results in no postoperative pain or reports of only mild pain.

The objective of other studies is to evaluate the modification by drugs of the neurohumoral, psychological and physiological responses to acute pain and apprehension in patients undergoing a stressful surgical procedure, the removal of impacted third molars. Prototype drugs employed include placebo, anti-anxiety agents, narcotic analgesics and barbiturates. The results of these investigations will clarify the role of these agents in the control of pain and apprehension as well as provide information on the interaction of these drugs with endogenous pain control systems.

Similar investigations are evaluating the effects of exogenous epinephrine administered with local anesthesia on cardiovascular and catecholamine responses to oral surgery. The response to surgical stress following oral surgery procedures was examined in a sample of patients who did not receive sedation nor epinephrine in their local anesthetic. No change was seen in cardiac output following local anesthesia administration which was accompanied by little change in circulating levels of epinephrine and norepinephrine. During the surgical procedure a 25% increase in cardiac output was seen which was accompanied by marked increases in circulating epinephrine and norepinephrine levels.

These findings indicate that the administration of local anesthesia without epinephrine does not result in appreciable changes in circulating catecholamines or cardiac output. The findings are in contrast to previous studies in which epinephrine administered in a local anesthetic resulted in a five-fold increase in circulating epinephrine levels as well as an increase in cardiac output.

A second sample of patients who were sedated with diazepam received on a random basis local anesthesia with or without epinephrine. In sedated subjects not receiving epinephrine, no change was seen in cardiac output or epinephrine levels during surgery. Norepinephrine levels were observed to decrease following diazepam sedation and then rise during surgery, to a level approximately equal to the preoperative level. In sedated subjects who received epinephrine, an increase in cardiac output was accompanied by a three- to four-fold increase in circulating epinephrine levels. These findings confirm our earlier observations that epinephrine administered

with local anesthesia is resulting in a marked increase in circulating epinephrine levels which is associated with measureable cardiovascular changes. These data also suggest that diazepam sedation results in an attenuation of the physiological arousal seen in unsedated patients, but that these effects are partially antagonized by administration of epinephrine with the local anesthetic.

These studies suggest that epinephrine included in local anesthetics is rapidly absorbed and results in measureable circulatory changes. While these changes are well-tolerated in our subjects who have been screened as healthy and free of systemic disease, they may not be so innocuous in the elderly or cardiovascular risk patient. Newer local anesthetics are sufficiently safe and of adequate duration to obviate the need for a vasoconstrictor in non-surgical procedures. The routine inclusion of epinephrine in local anesthetics may increase the potential for serious toxicity but without any increase in benefit to the patient.

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Anatomical studies of the trigeminal sensory nuclei and the spinal dorsal horn

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Gobel, Stephen	Chief, NEA Section	NA NIDR
Sugimoto, Tomosada	Visiting Fellow	NA NIDR
Humphrey, Emma L.	Bio. Lab. Tech. (Elec. Mic.)	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Neurocytology and Experimental Anatomy Section

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines the synaptic connections between primary neurons which make up trigeminal and spinal nerves on one hand and the neurons of the medullary and spinal dorsal horns on the other hand especially those in the substantia gelatinosa of Rolando. This project also considers the form, synaptic connections and function of the neurons of the dorsal horn as well as their pathological responses to peripheral nerve injuries and to the removal of central descending inputs. These studies employ electron microscopy, the Golgi method, degeneration techniques and the use of intraneuronal markers such as horseradish peroxidase and neurotoxins. The goals of these studies are to delineate trigeminal and spinal pain-temperature pathways and to broaden our understanding of oral-facial sensation.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00031-14 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Design and Computer Interfacing of Neurophysiologic Instrumentation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Brown, Frederick J.	Electronic Engineer (Instru)	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Neural Mechanisms Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These projects involve the development of suitable electronic and electromechanical instrumentation to be used in neurophysiological, physiological, and behavioral research. Electronic circuit design, using transistors, integrated circuits, and microcomputers, is used in the interfacing of these and other instruments to laboratory or multipurpose computer installations.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00132-08 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982 CT 0060102

TITLE OF PROJECT (80 characters or less)

Pharmacological Modification of Neurohumoral and Psychological Response to Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dionne, Raymond A.	Senior Staff Fellow	NA NIDR
Narrison, Michael S.	Expert	NA NIDR
Goldstein, David G.	Senior Staff Fellow	NE NHLBI
Wirdzek, Peggy R.	Clinical Nurse	NA NIDR
Clark, Barbara A.	Clinical Nurse	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Clinical Pain Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.90	.85

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is 1) to objectively evaluate the efficacy and clinical toxicity of drugs given to outpatients to alleviate apprehension associated with dental procedures, 2) to study the physiological, psychological and biochemical responses to the stress of dental therapy, and 3) to evaluate the role of exogenous epinephrine administered with local anesthetic on cardiovascular performance. Special attention has been given to the non-invasive measurement of cardiac output and stroke volume by thoracic impedance cardiography. A recent study employing this methodology indicates that exogenous epinephrine administered with local anesthesia results in a increase in circulating epinephrine levels and that there is a concommitant increase in cardiac output. A parallel investigation indicated that the elevated epinephrine levels and elevated cardiac output are not attenuated by diazepam premedication. Diazepam premedication does appear to suppress the elevation in circulating norepinephrine levels seen in non-sedated patients. These findings suggest that exogenously administered epinephrine results in an increase in circulating levels and a resultant increase in cardiac output.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00133-08 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982 CT 0060101

TITLE OF PROJECT (80 characters or less)

Assessment of experimental and clinical pain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Gracely, Richard H.	Research Psychologist	NA NIDR
Dionne, Raymond A.	Senior Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Duncan, Gary H.	Clinical Dental Assoc.	NA NIDR
Wolskee, Patricia J.	Psychologist	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Clinical Pain Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.10	.85	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are (1) to assess psychophysical methods of experimental pain measurement, i.e., magnitude estimation, category scaling, and cross-modality matching. Pain will be experimentally induced by electrocutaneous, electric tooth pulp, and mechanical heat stimulation; (2) to assess clinical pain measures, such as pain questionnaires and sensory matching methods, in a dental setting; (3) to determine the validity of experimental pain models by comparison of experimental and clinical pain responses; and (4) to evaluate known pharmacological and non-pharmacological pain-control agents.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00245-05 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982 CT 0060117

TITLE OF PROJECT (80 characters or less)

Sensations Produced by Tooth Pulp Stimulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dubner, Ronald	Chief, NAB	NA NIDR
Gracely, Richard H.	Research Psychologist	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Clinical Pain Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.20	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to determine the nature of sensations produced by tooth pulp stimulation. Non-pain, as well as pain sensations are evoked when low intensity electric current is applied to human teeth. In order to assess the role of non-pain sensations in the pulp—a traditionally exclusive pain system, these sensations were studied both psychologically and physiologically: 1) the minimum levels of current necessary to produce non-pain and pain sensations were determined for different frequencies of stimulating current; 2) the intensities of sensations from detection threshold to pain threshold were scaled by magnitude production and by verbal descriptors; 3) electromyographic (EMG) activity of the masseter inhibitory period was recorded during tooth pulp stimulation at both non-pain and pain currents; 4) the effect of a narcotic on sensations produced by tooth pulp stimulation and on the masseter inhibitory period was evaluated; and 5) the effects of a conditioning electrical stimulus, applied to the tooth, on sensation and on masseteric inhibitory period were determined.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00246-05 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

MPD Patients and Their Behavioral Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Heft, Marc W.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Clinical Pain Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.70	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of the present phase of this project are to compare: (1) aspects of illness behavior in myofascial pain dysfunction (MPD) patients and other chronic pain patients, and (2) the incidence of various signs and symptoms associated with MPD in MPD patients and in normals. Comparison between MPD patients to other chronic pain patients and normals will give insight into psychosocial factors which influence reports of MPD.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00247-05 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cytomorphology of functionally characterized spinal dorsal horn interneurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Bennett, Gary J.	Senior Staff Fellow	NA NIDR
Lu, Guo-Wei	Internat'l Res. Fellow	NA NIDR
Nishikawa, Nozomu	Visiting Fellow	NA NIDR
Ruda, Maryann T.	Senior Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Neural Mechanisms Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.70	3.20	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunocytochemical staining with enkephalin (ENK) antiserum revealed immunoreactive stalked cells in lamina II. Dorsal column postsynaptic spinomedullary neurons (DCPS) were retrogradely labeled with HRP in cats and monkeys. The lumbosacral enlargements of both species contained about 1,000 DCPS neurons. It is thus apparent that the DCPS system is one of the major sources of somatosensory input to the brain. Four types of DCPS neurons were characterized on the basis of the shape and orientation of their dendritic arbors. Electrophysiological analysis of antidromically identified DCPS neurons shows that about half respond only to tactile stimuli while the remainder respond also to noxious stimuli. Intracellular recordings show that many DCPS neurons respond to primary afferent input with an epsp-ipsp sequence. The ipsp is probably initiated by A-beta, low threshold mechanoreceptors. Its amplitude is frequency dependent in the range of 5-20 Hz. When retrogradely labeled DCPS neurons were immunocytochemically counterstained with a 5-HT antiserum, numerous 5-HT axonal varicosities were seen to contact the DCPS perikarya and proximal dendrites.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00276-04 NA

PERIOD COVERED: October 1, 1982 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Narcotic and Brain Stimulation Analgesia and Human Chronic and Experimental Pain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Gracely, Richard N.	Research Psychologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Dionne, Raymond A.	Senior Staff Fellow	NA NIDR
Noffert, Marvin J.	Senior Staff Fellow	NA NIDR
Wirdzek, Peggy R.	Clinical Nurse	NA NIDR
Wolskee, Patricia J.	Psychologist	NA NIDR
Lees, David E.	Deputy Chief	ANES CC

COOPERATING UNITS (if any)

Dr. Richard Greenberg, Division of Neurosurgery, Virginia Commonwealth Univ., Richmond, Virginia

Dr. Bruce Smoller, Psychiatric Consultant, 4400 East-West Highway, Bethesda, Maryland

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Clinical Pain Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.0	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) Assess the effectiveness of chronic electrical stimulation of midbrain sites for the relief of chronic pain in humans; (2) Evaluate the efficacy and mechanisms of traditional narcotic analgesia and compare these to chronic electrical stimulation of midbrain sites; (3) Validate experimental models of pain and their potential diagnostic use in chronic pain patients; and (4) Determine and compare the impact of both traditional narcotic and chronic electrical stimulation therapies on the functional, intellectual and emotional well being of these patients. Participants in this study will be (1) chronic pain patients receiving surgically implanted stimulating electrodes for pain control; (2) chronic pain patients maintained on traditional narcotic analgesics who will not receive implanted stimulating electrodes; and (3) healthy normal volunteers. The effects of chronic brain stimulation in surgical patients will be compared to the effects of narcotics previously administered to patients and to effects of narcotic regimes in nonsurgical chronic pain patients. In addition, the effects of narcotics on perceptual and neural mechanisms of experimentally induced pain will be assessed in pain-free volunteers.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00286-03 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982 CT 0060133

TITLE OF PROJECT (80 characters or less)

Evaluation of Oral Analgesics for Ambulatory Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dionne, Raymond A.	Senior Staff Fellow	NA NIDR
Wirdzek, Peggy R.	Nurse Specialist	NA NIDR
Narrison, Michael B.	Expert	NA NIDR
Gracely, Richard H.	Research Psychologist	NA NIDR
Butler, Donald P.	Sr. Staff Den (Oral S)	CIPC NIDR
Fox, Philip C.	Dental Officer	CIPC NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Clinical Pain Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.75	1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project consists of studies (1) to evaluate the efficacy of two novel pain control agents, flurbiprofen and etidocaine, when given in combination to suppress postoperative pain; (2) to evaluate the anti-inflammatory efficacy of flurbiprofen in comparison to injectable steroids; (3) to evaluate the analgesic efficacy of a novel narcotic antagonist analgesic, codorphone, in comparison to a standard narcotic, codeine. The combination of flurbiprofen, a non-steroidal anti-inflammatory drug, and etidocaine, a long-acting local anesthetic, was demonstrated to result in a significant suppression of postoperative pain compared to standard therapy. This clinical efficacy represents a genuine therapeutic advantage in that fewer side effects were reported following the experimental therapy. This combination possesses potential for improvement in the treatment of post-operative pain in that one-third of the sample reported no pain following the surgical removal of impacted molars, a procedure which normally results in moderate to severe postoperative pain.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00288-03 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Neuropharmacological Characterization of Synaptic Circuitry in the Dorsal Horn

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ruda, Maryann T.	Senior Staff Fellow	NA NIDR
Coffield, Julie Ann	Biological Lab Tech (Micro)	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Neural Mechanisms Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.30	.70	1.60

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunocytochemical localization of neurotransmitters at the light and EM level was used to determine the potential functional role of the neurotransmitter in the neural circuitry of the medullary and spinal dorsal horn.

The opiate peptide enkephalin was shown to modulate the transmission of nociceptive information through synapses on thalamic projection neurons in both laminae I and V. This observation demonstrates that opiates act on post-synaptic receptors, located on thalamic projection neurons.

Serotonin immunoreactive axonal endings were identified at the light and EM level. They were found in all laminae of the dorsal horn. In the superficial layers, serotonin axons oriented rostro-caudally. Serotonin endings synapsed mainly on dendrites and occasionally on cell somata. Descending serotonin modulation thus acts predominately through synapses on intrinsic dorsal horn neurons.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00291-03 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Neural Correlates of Behavior in the Monkey Medullary Dorsal Horn

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Duncan, Gary H.	Clinical Dental Assoc	NA NIDR
Bushnell, Mary C.	Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Ne, Lien-Fang	WHO Internat'l Fellow	NA NIDR
Taylor, Mark B.	Animal Caretaker	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Neural Mechanisms Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.95	1.90	1.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project studies the effect of behavioral and environmental variables on responses of thalamic projection neurons in the medullary dorsal horn (trigeminal nucleus caudalis) to noxious and innocuous thermal stimuli. Rhesus monkeys are trained to detect the termination of innocuous heat stimuli and the onset of noxious heat stimuli. In a second task, these monkeys detect the onset of a light stimulus. Trigeminothalamic neurons code thermal discriminative information used by the monkey within the behavioral tasks. Some neurons that respond to passive mechanical and thermal stimulation also respond to other stimuli the monkey uses for successful completion of the task. We observe several patterns of task-related activity, and some neurons showing each pattern project to the thalamus. These task-related responses may modulate sensory activity and thereby influence the perception of and response to oral-facial pain.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00312-02 NA

PERIOD COVERED: October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunocytochemistry of Identified Spinal Dorsal Horn Laminae I and IIa Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Noffert, Marvin J.	Senior Staff Fellow	NA NIDR
Miletic, Vjekoslav	Postdoctoral Fellow	NA NIDR
Ruda, Maryann T.	Senior Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Neurobiology and Anesthesiology Branch

SECTION: Neural Mechanisms Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.85	2.65	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Neurons in lamina I and II of the lumbar spinal cord of the cat were characterized physiologically in terms of responsiveness to various natural stimuli (such as pinch and brush), peripheral input (C fiber vs. A-delta vs. A-beta), and effects of nucleus raphe magnus stimulation. These neurons were then intracellularly injected with horseradish peroxidase, the cats perfused, and the spinal cord sectioned and reacted with diaminobenzadine. The neurons were identified, the tissue processed immunohistochemically with antibodies to serotonin and substance P, and the cells and immunoreactive boutons then drawn by camera lucida technique with high power light microscopy.

PHS-6040 (Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00313-02 NA

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Conjoint Measurement Analysis of Metric and Nonmetric Stimuli

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Heft, Marc W.	Postdoctoral Fellow	NA NIDR
Gracely, Richard N.	Research Psychologist	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this study are to assess the contributions of category meanings and positions on category lists on the perceptual scale values for those items. Subjects rate differences between stimulus pairs consisting of an electric shock intensity and a word or category number from three distinct seven-point category lists. Perceptual scale values for both the electric shock and category items are determined by Conjoint Measurement analysis. Comparisons of the derived exponents for Stevens' power functions for electric shock show that subjects are performing the tasks similarly in three experiments, so comparison of the perceptual scales for the category items is appropriate. Results from these comparisons show that category meanings rather than positions are important in determining perceptial scale values. In addition, subjects estimate the magnitude of sensations evoked by electrical stimulation. Power functions also describe the relation between the numerical judgements and currents. In almost all instances the derived exponents for electric shock determined in the difference estimation experiment were less than those determined in the single-stimulus experiments, consistent with results from other perceptual continua.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00314-02 NA

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Effect of morphine on experimental and clinical pain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Wolskee, Patricia J.	Psychologist	NA NIDR
Gracely, Richard N.	Research Psychologist	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Neurobiology & Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.60	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this study are: 1) to determine the effect of morphine on the psychophysical judgments of sensory intensity and unpleasantness responses to clinical and experimental pain in normal subjects and chronic pain patients and 2) to determine the validity of experimental pain models by comparison of experimental and clinical pain responses.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00329-01 NA

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Discrimination of Thermal Stimuli Applied to the Face in Monkey and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Bushnell, Mary C.	Staff Fellow	NA NIDR
Duncan, Gary H.	Clinical Dental Assoc	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Taylor, Mark B.	Animal Caretaker	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Neurobiology & Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.05	1.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project studies the ability of rhesus monkeys and humans to discriminate small differences in innocuous warm pulses and noxious heat pulses applied to the face. It also evaluates the influence of atrention on thermal discriminative ability. Both monkeys and humans are better able to discriminate small differences in noxious heat pulses (47°C) than innocuous warm pulses (39°C). Since primary afferent warm fibers provide as much differential temperature information as do thermal nociceptors, this difference in performance must be centrally mediated. Attentional state appears to influence the ability to detect small temperature changes on the face. A signal correctly indicating the location of a subsequent thermal change improves detection performance while an incorrect signal worsens performance. Consequently, attentional factors may influence the perception of and response to oral-facial pain.

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